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BIOCHEMISTRY AND MORPHOGENESIS

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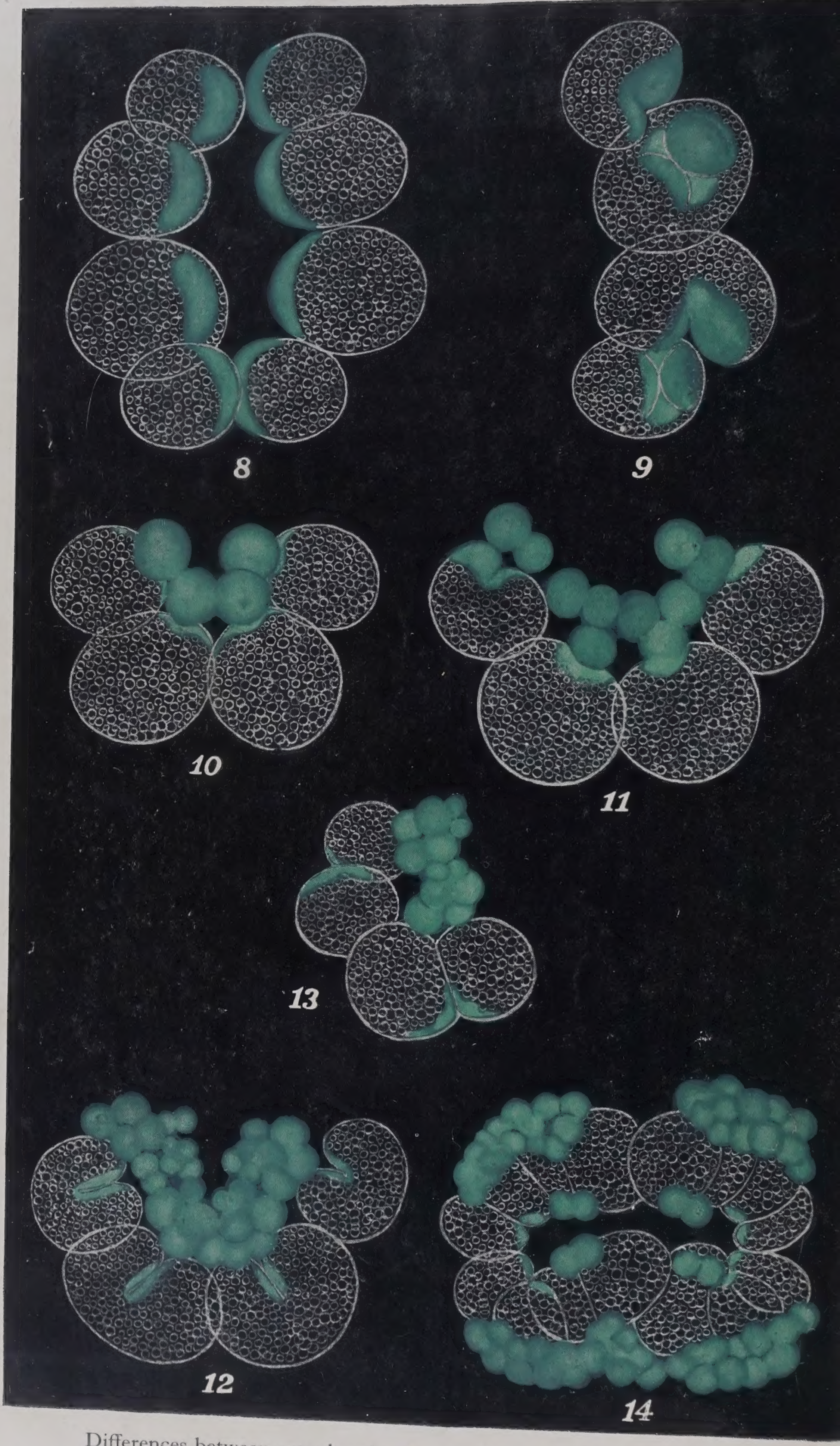
Amphibian development,
Genes,
Metabolism,
Cell differentiation
" growth.

Frontispiece



Differences between cytoplasmic cortex and interior in a ctenophore egg seen under dark-ground illumination (*Beroe*), see Fig. 49.

BIOCHEMISTRY AND MORPHOGENESIS

by

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Cambridge

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孟子曰
博學而
詳説之
將以反
説約也

Mencius said: *extensively learn and in all detail state it, so that later, summarise its essence.*

4th century B.C.

此書紀念
友人
李大斐
魯桂珍
女士

李約瑟

NOTE ON ILLUSTRATIONS

Of the 328 illustrations in this book a number (including 4 in colour) are printed as plates and inserted as follows:

Frontispiece
Plate I *facing page* 42
II 46
III 132
IV 146
V 154
VI 155
VII 158
VIII 160
IX 166
X 168
XI 172
XII 174
XIII 176
XIV 177
XV, XVI, XVII, XVIII *between* 178 & 179
Plate XIX *facing page* 190
XX 234
XXI 274
XXII 278
XXIII 290
XXIV 294
XXV 300
XXVI 301
XXVII 342
XXVIII 358
XXIX 360
XXX 362
XXXI 372
XXXII 438
XXXIII 464
XXXIV 496
XXXV 674

CONTENTS

PAGE

GENERAL INTRODUCTION xiii

PART 1. THE MORPHOGENETIC SUBSTRATUM

1·05 Introduction 3

1·1 Problems of maturation 3

1·2 Constitution of the eggs of birds 6
- 1·21 The shell and shell-membranes 7
- 1·22 The white 7
- 1·23 The vitelline membrane and its physiology 11
- 1·24 The yolk 14
- 1·25 Inorganic constituents 17

1·3 The effect of pre-developmental factors on development 20

1·4 Constitution of the eggs of lower vertebrates and invertebrates 24
- 1·41 Reptiles and Amphibia 24
- 1·42 Fishes 25
- 1·43 Arthropods 27
- 1·44 Echinoderms 28
- 1·45 Molluscs and Worms 29

1·5 The inheritance of immunity 30

1·6 The egg and the environment 32
- 1·61 The problem of protection; larval compression and ovoviviparity 32
- 1·62 The evolution of the cleidoic egg 33
- 1·63 The direct transition from the non-cleidoic state to ovoviviparity 38
- 1·64 Metabolic properties of the cleidoic state 48

1·7 Standard mortality curves 63

1·8 Embryonic nutrition 66
- 1·81 The scaffolding of the chick embryo 66
- 1·82 Yolk utilisation in the lower vertebrates and invertebrates 73
- 1·83 Avian "milk" and mammalian colostrum 75
- 1·84 Maternal diet and embryonic constitution in mammals 77

1·9 The mammalian placenta 79
- 1·91 Placental constitution 85
- 1·92 Placental metabolism 89
- 1·93 Placental transmission 90
- 1·94 The amniotic and allantoic liquids 93

PAGE

PART 2. THE MORPHOGENETIC STIMULI

- 2·05 Introduction 97
- 2·1 General concepts of causal morphology 98
  - 2·11 Determination 99
  - 2·12 Morphogenetic stimuli 103
  - 2·13 A digression on plant galls 106
  - 2·14 The hierarchy of organisers in animal development 110
  - 2·15 Competence and unstable equilibrium 112
  - 2·16 The liquidation of the entelechy 119
  - 2·17 Regional differentiation 124
  - 2·18 Field phenomena 127
  - 2·19 Conclusion 130
- 2·2 Mosaic eggs and their biochemistry 131
- 2·3 Amphibian development and the fundamental principles of morphogenesis 141
  - 2·31 Description of gastrulation 141
  - 2·32 The mapping of the presumptive regions 143
    - 2·325 The nature of the formative movements 145
  - 2·33 The appearance of the vertebrate axis 148
    - 2·331 Properties of the primary organiser 148
    - 2·333 Necessity of the primary organiser 156
    - 2·335 Determination of the endoderm and mesoderm 163
  - 2·34 Biochemistry of the primary organiser 165
    - 2·345 Chemistry of the primary organiser 176
  - 2·35 Morphogenesis and metabolism 188
    - 2·351 Metabolism of the regions of the gastrula 188
    - 2·352 Respiration and Glycolysis in the regions of the gastrula 193
    - 2·353 The role of sulphydryl in gastrular metabolism 203
    - 2·355 The nature of the inactive evocator complex 205
    - 2·356 The evocator compared with plant hormones 212
    - 2·357 Induction and fertilisation 214
  - 2·36 Blastopore formation and the liberation of the evocator 216
  - 2·37 Anomalies of organiser function 222
    - 2·371 Organiser inhibition 222
    - 2·375 Organiser excess; Twinning 228
    - 2·376 Organiser excess and anomalous competence; Teratomata 231
  - 2·38 Organisers and cancer 239
    - 2·381 The escape from the individuation field 239
    - 2·383 Evocators, carcinogens and oestrogens 243
    - 2·384 The nature of biological specificity 261
    - 2·385 Homoiogenetic induction and cancer 267

PAGE

2·39 Regional differentiation 271
  2·391 Evocation and individuation 271
  2·392 Individuation as the organised action of eidogens 278
  2·393 Contiguity and affinity effects 284
  2·394 Individuation and the organ-districts 286
  2·395 Second-grade organisers 290
    2·3951 Eye-cup and lens induction 291
    2·3952 Other second-grade and third-grade inductors 301
    2·3953 Limb-bud induction 305
    2·3954 Opercular perforation 308
  2·396 Second-grade organisers and caste differentiation in social animals 309
  2·397 Sex determination 310
  2·399 Organisers and recapitulation 319

2·4 Organisers and fate-maps in Prochordates, Fishes, Sauropsida and Mammals 320
  2·41 A comparison of fate-maps 321
  2·42 Determination in Prochordates 325
  2·43 Determination in Cyclostomes and Fishes 325
  2·44 Determination in Birds 330
  2·45 Determination in Mammals 336

2·5 Temperature and morphogenetic processes 336

2·6 Genes and organiser phenomena 340
  2·61 The action of organisers across genetic boundaries 341
  2·62 Genes and the later stages of individuation 343
  2·63 The origin of species-specificity 348
  2·64 The role of the nucleus in development 351
    2·645 Merogons and chimaerae 357
  2·65 Lethal genes and organisers 365
    2·651 Genes causing the death of the embryo before the action of the primary organiser 367
    2·652 Genes interfering with the action of the primary organiser 368
    2·653 Genes interfering with the action of second-grade organisers 375
    2·654 Genes producing anomalies of the individuation field by changes in the growth rates of parts 379
      2·6545 Hereditary tumours 385
    2·655 Genes producing anomalies of embryonic metabolism 387
    2·656 Genes killing or deforming the embryo by unknown mechanisms 391
  2·66 The kinetics of gene action 391
    2·661 Phenocopies and rate genes controlling morphological processes 392
    2·662 Phenocritical periods 402
    2·663 Rate genes controlling chemical processes 405
  2·67 Nuclear inductors and nuclear metabolism 408
  2·68 The control of embryo and adult size 420

PAGE

2·7 Problems of later amphibian life-history 430
  2·71 Regeneration, determination, and the organ-districts 430
  2·72 Biochemical aspects of regeneration 442
  2·75 Metamorphosis, competence, and the last inductors 447

2·8 Determination in insect development and metamorphosis 455
  2·81 Organiser phenomena in insects 456
  2·82 Post-embryonic inductors in insects 463
  2·85 The biochemistry of insect metamorphosis 470

2·9 Determination in echinoderm development and metamorphosis 476
  2·91 The double-gradient system 477
  2·92 Experimentally induced deviations from presumptive fate 480
  2·93 Chemically induced deviations from presumptive fate 485
  2·94 Combinations of the experimental and chemical methods 488
  2·95 A critique of the chemical aspects of echinoderm determination 492
  2·96 Later determinations 497
  2·97 Organiser phenomena in echinoderms 500

PART 3. THE MORPHOGENETIC MECHANISMS

3·05 Introduction 505

3·1 Dissociability 505
  3·11 Disengagement of growth and differentiation 505
    3·111 Growth without differentiation 507
    3·112 Differentiation without growth 507
    3·113 Nuclear division without cell-division 508
    3·114 Cell-division without nuclear division 509
    3·115 Differentiation without cleavage 509
    3·116 Differential action of hormones, amino-acids, etc. on differentiation and growth 513
    3·117 Disengagement of determination from differentiation and growth 513
    3·118 Mutual independence of growth, histogenesis and organogenesis 514
  3·12 Disengagement of growth, differentiation and metabolism 514
    3·121 Metabolism without growth 514
    3·122 Metabolism without growth and differentiation 516
    3·123 Stepwise inhibition of growth, differentiation, fermentation and respiration 517
    3·124 Cell-division and differentiation without oxidative metabolism 522
    3·125 Selective engagement of growth or differentiation and characteristic chemical activity 523
    3·126 Disengagement of growth or differentiation from characteristic chemical composition 524
  3·13 Dedifferentiation and degrowth 525
  3·14 Integration or engagement of the fundamental processes 529

PAGE

3·2 Heterauxesis 531
  3·21 Embryonic growth in general 531
  3·22 The theory of heterauxesis 532
  3·23 Chemical heterauxesis 540
  3·25 The chemical ground-plan of animal growth 558

3·3 Respiration 562
  3·31 Respiration of the eggs of Echinoderms, Worms and Molluscs 562
  3·32 Respiration of the eggs of Arthropods 577
  3·33 Respiration of the eggs of Fishes 584
  3·34 Respiration of the eggs of Amphibia 587
  3·35 Respiration of the eggs of Sauropsida 590
  3·36 Respiration of mammalian embryos 595
    3·365 The rise and fall of metabolic rate 600
  3·37 The succession of energy-sources 603
  3·38 Respiratory enzymes 603
  3·39 Axial gradients and respiration 605

3·4 Metabolism 605
  3·41 Indirect approaches 606
  3·42 Carbohydrate metabolism 607
    3·421 Carbohydrate metabolism in the development of higher vertebrates 607
    3·422 The phosphorylation problem 610
    3·423 Ascorbic acid 616
    3·424 The fructose problem 616
    3·425 Carbohydrate metabolism in the development of lower vertebrates and invertebrates 617
  3·43 Protein metabolism 619
    3·431 Protein metabolism in the development of higher vertebrates 619
    3·432 Protein metabolism in the development of lower vertebrates and invertebrates 624
    3·433 Hatching proteases 627
    3·434 The growth-promoting factor 628
  3·44 Nuclein metabolism 632
  3·45 Lipin and sterol metabolism 637
    3·455 Choline esterase and the development of nervous function 642
  3·46 Pigment metabolism 643
    3·461 Pyrrol derivatives 644
    3·462 Lipochromes 652
    3·463 Flavines 654
    3·464 Pterines 655
    3·465 Melanin 655

PAGE

3·5 Polarity 656
 3·51 The organisation of cell-protoplasm 656
 3·52 Fibre-molecules and "dynamic structure" 658
 3·53 The paracrystalline state 661
 3·54 Rigidity in paracrystalline mesoforms and in embryonic structures undergoing determination of spatial axes 664
 3·55 Modifications of polarity by external influences 667
 3·56 Molecular orientation and contractility as the basis of response to morphogenetic inductors 670
 3·57 Anisometric protein molecules in eggs and embryos 671
 3·58 The mechanism of gastrulation 673
 3·59 The fibres of the mesoderm 674
  3·595 Dynamic structure at the molecular level 676

CONCLUSION 679

GLOSSARY OF SOME OF THE SPECIAL TERMS USED IN THIS WORK 681

BIBLIOGRAPHY 691

GENERAL INDEX 769

INDEX OF ANIMALS 781

INDEX OF PLANTS 786

INDEX OF GENES 787

Bibliographical conventions

Where only one paper or group of papers by an author or group of authors is listed the text contains nothing but the name or names. Where more than one paper or group of papers is referred to, superscript figures are added to the names in the text to allow of identification.

The use of the shortened and (&) indicates collaboration between two or more authors.

Conventions adopted in the chemical formulae

(1) No carbon atoms or their associated hydrogens are shown. Bonds of full length have C at each end, except where other elements occur heterocyclically, or by analogy heterocyclically; such atoms are shown as part of the ring, as if they were C.

(2) Terminal CH_2 is shown simply by the ending of the chain of bonds. Terminal COOH is shown as OOH.

(3) In all chains of bonds a C atom occurs at every angle, together with sufficient H to satisfy its valencies.

(4) It will be seen that many non-cyclic compounds are written as if they were composed of incomplete rings open at one point, rather than elongated chains; in some cases (e.g. vitamin D_2) there is definite evidence for this formulation, but in most cases (e.g. squalene) it is theoretical.

(5) All unsaturated linkages are shown as such.

GENERAL INTRODUCTION

There are three ways in which the great problem of the relation between morphology and biochemistry—the problem to which this book is devoted—can be approached. In the first place we may make a direct attack upon that difficult region lying between the largest chemical particles and the smallest morphological structures which we know. This realm includes the study of paracrystalline aggregates, colloidal micelles, fibrous macro-molecules, protein structure, etc. In recent times the physico-chemical study of the viruses has played a part of cardinal importance in exploration along these lines. The second way in which we may attempt to bridge the gulf between morphology and biochemistry is by studying the chemical changes which go on during embryonic development, a time during which the morphological change is the most obvious variable.

Up to 1931, in which year the author's former book *Chemical Embryology* was published, this was perhaps the only contact between biochemistry and embryology, and the chemist could have relatively little to say about real morphogenesis itself. But it was in that very year that successful embryonic inductions were first obtained after the destruction of the living integrity of the primary organisation-centre of amphibia. Hence there opened the possibility of a third method of approach; the biochemical investigation of what may truly be called the morphogenetic hormones. The study of these chemical substances, of fundamental importance for all animal and plant development, including its hereditary aspect, occupies the greater part of the present book. The establishment of the existence of such substances, the essential elements in the processes of dependent differentiation, known since the time of Wilhelm Roux, took approximately the first twenty years of the present century and will always be associated with the names of Ross G. Harrison, Warren Lewis, Hans Spemann and Otto and Hilde Mangold. The second twenty years of the century, which may come to be spoken of as the period between the two great European wars, were characterised by numerous attempts to isolate the inductor substances and to understand their action. Although much has been achieved, as will be evident from the contents of the present book, we have come to see that the problems are often far more complex than had at first been thought, and that after all the larger part of the mystery remains in that we can as yet form little idea of what constitutes reactivity—the competence to react to the morphogenetic inductor.

In accordance with this distribution of interest, therefore, the present book is divided into three parts. In Part 1 the morphogenetic substratum is considered, i.e. the chemical raw material of development and all the manifold nutritional problems which embryos have solved. Part 2 is devoted to the morphogenetic stimuli themselves, and Part 3 to the morphogenetic mechanisms. Under this head, besides the special metabolism of embryonic life, such general questions as dissociability, heterauxesis, the basis of polarity, etc. are discussed.

Before entering upon the details, however, there are two theoretical questions to which a reference must be made. The relation of biochemistry to morphology is not a banal matter. It has been implicit in countless discussions of theoretical biology and involves themes which have run right through the history of philosophy from the earliest speculations of Greek and Chinese thinkers through the sixteenth, seventeenth and eighteenth centuries, down to our own time. Here I wish only to state my position with regard to two of these, the "irreducibility" of the biological, and the question of form and matter.

Morphologists, turning over the pages of the present book, will probably be inclined to remark that the author finds only "a mass of substances" in the living and developing organism. Yet that is precisely what the organism is, containing in itself innumerable molecules of small size some of which are indeed the morphogenetic inductors, as well as the giant protein molecules which, in responding by new orientations, form the basis of the cellular and organic architecture. Nevertheless the biochemist approaching morphological problems does not forget that all these molecules are ordered and organised in a totally different way to anything which occurs outside living organisms. His difficulties arise rather from the vagueness and lack of quantitative precision common to so much morphological work and perhaps inseparable from it. But everything that can be done to remove this vagueness is being done, and after all, we have already a great many extremely well-defined histological and morphological factors which can be correlated with biochemical ones. Some may feel that the terminology of inductors, competences, etc., is too "cut and dried", but the history of science abundantly shows that in every age, whether of the "word-rectification" school in ancient China, the Arabs, Albertus Magnus, the early Royal Society, or the time of Haller and Boerhaave, the invention and clarification of terms is one of the greatest limiting factors in scientific advance. A clear formulation, even though partial, is better than a vague one; and we must not, in avoiding rigidity, fall into obscurantism. Any who might be led to suppose that words such as "inductor", "evocator", etc., carry animistic undertones, would be under a complete misapprehension. They stand for definite chemical substances the nature of which is as yet not fully elucidated. They conceal no *archaeus* or demon. In the use of the phrase "oxidising agent" the chemist risks no confusion with "passenger agent", and "hydrogen-donators" head no subscription-lists. In this book, the word "organiser" is always used to imply the presence of tissue, whether living or dead; "evocator" refers only to a specific chemical substance; and "inductor" is a looser word available for both these senses. The glossary of definitions on p. 681 will perhaps assist the reader's mental labour. It makes no legislative claims, but only seeks to show the exact senses in which terms are used in the present book, and it ends with a small group of words which now deserve their *requiescat*.

In the previous discussion the words "organised" and "ordered" are the keynotes. We cannot but consider the universe a series of levels of organisation and complexity, ranging from the sub-atomic level, through the atom, the molecule, the colloidal particle, the living nucleus and cell, to the organ and the organism, the psychological and sociological entity. It follows that the laws or regularities which we find at one level cannot be expected to appear at lower levels. The conditions for their appearance do not exist there. It is no use fishing in homogeneous solutions for the laws which hold good for the behaviour of liquid crystals, still less for those which hold for that of living organisms.*

Philosophers have often talked about the reducibility or irreducibility of biological facts to physico-chemical facts. These old controversies are unnecessary if we realise that we are dealing with a series of levels of organisation. We must seek to elucidate the regularities which occur at each of these levels without attempting either to force the higher or coarser processes into the framework of the lower or finer processes, or conversely to explain the lower by the higher. From this point of view, the regularities discovered by experimental morphology will always have their validity and will, in a sense, be unaffected by anything which either biochemistry on the one hand, or psychology on the other, may discover. The behaviour, for example, of an embryonic eye-cup isolated into saline solution, its capacities for self-differentiation, fusion with another eye-cup, lens-induction, regulation, etc., etc. will always remain the same however much our knowledge of biochemistry or biophysics may advance. This is the reason why prediction is possible at a level of organisation which, strictly speaking, we do not yet understand at all, for instance, genetics. But the important point is that although the regularities established at the level of experimental morphology are irrefragable, they will, in the absence of biochemical experimentation, remain for ever meaningless. Meaning can only be introduced into our knowledge of the external universe by the simultaneous prosecution of research at all the levels of complexity and organisation, for only in this way can we hope to understand how one is connected with the others.

This brings us to the ancient distinction between *forma* and *materia*, mainly due to the analytical mind of Aristotle—*μορφή* and *εἶδος* against *ὕλη*. This distinction has had an influence incalculably great on the historical development of biology.† Morphologists for many centuries past have devoted themselves to the study of living form without much consideration of the matter with which it is indissolubly connected. It is not surprising that the numerous devils of vitalism found a congenial abode in the mansions of empty *forma* thus suitably swept and garnished. Moreover, the morphological tradition‡ was to think of matter much too simply, ignoring the vast complexity of chemical structures and the unbroken line of sizes reaching from the hydrogen atom at one end (to say nothing of sub-atomic levels) to the virus molecule at the other. Only in the light of the conception of integrative levels can the saecular gulf between morphology and chemistry be bridged.

---

* For a fuller discussion of integrative levels, see the present author's Herbert Spencer lecture at Oxford, *Integrative levels; a revaluation of the idea of Progress*. The general picture there drawn has relations with evolutionary naturalism, emergent evolutionism, and dialectical materialism.

† For a brilliant summary of Aristotle's views on these subjects, with convenient references to the texts themselves, see W. D. Ross' "Aristotle".

‡ Originating from the conception of becoming as the privation of one form and the donation of another.

It is true that Aristotle held that there could be form without matter, thoug no matter without form. But according to him, the only entities which possessed form without matter, were the divine prime mover, the intelligent demiurge that moved the spheres, and perhaps the ψυχῆς διανοητική (the rational soul of man. Some of these are factors in which experimental science has never been very much interested. On the other hand, he maintained that there could be no matter without form, for however pure the matter was (even the chaotic prima menstrual matter which was the raw material of the embryo), it was always composed of the elements, that is to say, it was always hot, cold, dry or wet, and hence had a minimum of form. In its primitive way this mirrors the standpoint of modern science. Form is not the perquisite of the morphologist. It exists as the essential characteristic of the whole realm of organic chemistry, and cannot be excluded either from "inorganic" chemistry or nuclear physics. But at that level it blends without distinction into order as such, and hence we should do well to give up all the old arguments about form and matter, replacing them by two factors more in accordance with what we now know of the universe, that is to say, Organisation and Energy. From this point of view there can be no sharp distinction between morphology and biochemistry, and we may have every hope that in the future we shall be able to see not only what laws the form of living organisms exhibits at its own level, but also how these laws are related to those which appear at lower and higher levels of organisation.

I terminate this introduction, as in private duty bound, by an expression of my deep indebtedness to all those who have helped me, whether in the train of thought that led to this book, or with specific problems arising out of it. First, to my colleagues and all my collaborators, of many countries and peoples. Secondly, to all my friends in the New World, Americans either by birth or adoption, especially those who gather in the Symposia on Differentiation and Growth; inheritors now of both the two greatest national traditions in causal morphology. From all these have I learnt, and without their discussions many a problem would for me still lie in obscurity. Lastly, to that ancient and unique foundation, the Cambridge University Press, with which I am proud to be connected, and where my friends in every department spare no effort to make a book such as this all that it should typographically be.

Part One

THE MORPHOGENETIC SUBSTRATUM

THE MORPHOGENETIC SUBSTRATUM

1·05. Introduction

In *Chemical Embryology** an attempt was made to summarise all existing knowledge regarding the chemistry of the developing embryo and the raw materials from which it is formed.† Ten years ago this summary was inevitably restricted to the actual chemical constitution of the embryo and the raw materials, and to what was known of the metabolism during embryonic development. To-day the interest has shifted to the analysis of the fundamental morphogenetic stimuli which operate in embryonic life. These questions form the subject-matter of Part 2. But it is indispensable to precede them by an account of the main advances during the past ten years in our knowledge of the raw materials from which the embryo is built, and to follow them by a similar account of the new knowledge of embryonic growth and metabolism. These are the subjects of Parts 1 and 3 respectively.

1·1. Problems of maturation

A satisfactory knowledge of the biochemistry of the egg would include not only an exact understanding of the part which each chemical constituent of the egg has to play in the development of the embryo, but also an understanding of the way and order in which these constituent substances are laid down in the preparation of the egg in the ovary. It is likely that without this understanding we shall never be able to comprehend the real nature of those properties of polarity and symmetry for which the egg before development is so remarkable (see Section 3·5). But at present we are far from such a complete knowledge, and we have to content ourselves with fragments of information which we may hope to fit together better later.‡

Most of the work on the maturation§ of the egg has been of a histochemical nature, and of that which has been done with direct chemical methods the greater part concerns the eggs of birds, especially the hen. We shall therefore begin this section with the hen's egg. Like all the others in Parts 1 and 3, it should be read in conjunction with the earlier discussions in *CE*.

The changes occurring in the blood stream of the hen are very considerable. They have been summarised by Riddle[2]. Blood-sugar rises 25 %, blood-calcium 100 %, blood-fat 35 %, and ether-soluble phosphorus 50 %. The weight of the adrenals increases by about 40 % and the oviducts hypertrophy 800 %. Huxley[10] has shown that it is possible to correlate these large physiological variations which follow the rather sudden change from slow to rapid growth of the oocyte (confirmed by Chomković and Orrù[9]) with studies on bird behaviour. Jukes & Kay[3], in their review on yolk proteins, speculated on the mechanism of the rapid deposition of vitellin, pointing out that 99 % of the 3 gm. of protein in an egg-yolk is secreted during the last 5 to 8 days before laying. This protein may be produced from the amino-acids in the plasma or possibly from the plasma proteins by some much less drastic change. Rimington noted that in their distribution of nitrogen (as determined by Crowther & Raistrick) casein and lactoglobulin (serum globulin) are not remotely dissimilar, and himself showed that artificially phosphorised serum globulin has several similarities to casein. Laskowski, in a series of papers, subsequently described the isolation of vitellin from the serum, not only of laying birds, but also of the tortoise and the trout. It would seem, therefore, to be formed in some organ as well as in the ovary. According to Schenck's[2] van Slyke analyses the composition of vitellin from young oocytes differs from that of fully mature eggs, as it contains a higher proportion of hexone bases. Serum vitellin is serologically identical with ovovitellin (Roepke & Hughes; Roepke & Bushnell). After a single dose of pituitary gonadotrophic hormone, serum vitellin appears in the blood (Laskowski); after further administration serum calcium is increased (Riddle & Dotti; Hughes, Titus & Smits), and the ovary greatly hypertrophied (Bates, Lahr & Riddle). The calcium increase is in the non-ultra-filtrable fraction (Correll & Hughes).

---

* Hereinafter referred to as *CE*.

† Since the publication of *CE*, reviews covering the whole or parts of the field have been published as follows: (1934) Paechtner; Ermakov; (1935) Grossfeld; (1936) Ogorodny. Reviews dealing with specialised problems will be referred to in what follows.

‡ I exclude from consideration here the nuclear phenomena of maturation, the reduction divisions, etc., as they are fully discussed in genetical books and monographs.

§ Cf. the brief review of van Dyke.

By a new method, the administration of labelled (radioactive) sodium phosphate to hens by subcutaneous injection, it has been found that all the lecithin of the yolk is taken up from the plasma, the plasma lecithin being replaced by molecules formed at least mainly in the liver (Hahn & Hevesy; Hevesy & Hahn). It is clear that the variations in the blood of the laying oviparous vertebrate are so large that serologically specific qualities of the blood should be expected during egg-laying; these have been observed by Sasaki on fowls.

During the early, slow, growth phase, the oocyte has a much higher water-content than later on (Romanov[4]), and during the late maturation phase fat and calcium are rapidly deposited, but approximately proportionally. The excess phosphate excretion shown by Common to occur during laying is ascribed by McGowan to the large deposition of calcium as carbonate in the shell during the passage of the egg down the oviduct. What happens to the egg after experimental surgical interference with the various parts of the oviduct has been described in the interesting paper of Asmundson[1], to which the reader must be referred for further details. The view previously held (see *CE*, p. 235), that an internal secretion of the thymus ("thymovidin") is responsible for the proper formation of the shell, cannot yet be taken as proved, since effective thymus extirpation, owing to the widespread presence of masses of lymphoid tissue which may have the same function, cannot be carried out (Greenwood & Blyth; Riddle & Křížinecký). There are valuable reviews on the factors affecting egg number (Fauré-Fremiet & Kaufman) and egg weight (Graham), and the whole subject of the formation of the hen's egg has been reviewed by Conrad & Scott.

The layers of white yolk which alternate with those of yellow yolk in the hen's egg-yolk* (see *CE*, p. 286) are said to be due to a deficiency of nutrient materials in the blood stream between 1 and 5 a.m., but they depend to some extent on the nature of the diet of the laying hen, as shown by Conrad & Warren (cit. in Conrad & Scott). Warren & Scott have found that a yolk enters the oviduct about 15 min. after ovulation, passes through the *infundibulum* or funnel in 18 min., spends 3 hr. in traversing the *magnum* or albumen-secreting portion of the oviduct, 1 hr. in the isthmus, and the remainder of the interval, usually 20 to

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* As is well known they are demonstrable by the ring-like deposition of dyes, which when added to the maternal diet enter the eggs. This has been done on the silkworm (Jucci & Ponseveroni) as well as the hen (Riddle[1]).

24 hr., in the uterus. Peristalsis as well as ciliary action aids the descent of the egg. In the magnum the egg-white is secreted round the yolk as a dense homogeneous gel; this is a network of mucin fibres (R. K. Cole) enclosing a solution of the other egg-white proteins (ovoalbumen, conalbumen, and ovomucoid). Much of our knowledge of the formation of the white and shell has been found out by operations on laying fowls, the details of which may be had in Asmundson & Burmester; Burmester, and Asmundson & Jervis.

The nature of the chalazae has always been mysterious (see *CE*, pp. 117, 135). While Richardson attributes their formation to a special region of the oviduct, Conrad & Phillips explain it as follows. The innermost layer of the magnum gel becomes liquefied as the egg passes down the oviduct, so that the yolk is free to rotate in it. "While it is in the uterus the egg is rotated about its long axis, and since the yolk always tends to float with the blastoderm up, the white is rotated round the yolk. This action wraps the mucin fibres around the yolk and twists them at the ends to form the chalazae. Removal of the mucin fibres from the inner layer of gel liquefies it, and it becomes the inner thin white" (see below, p. 7). Unfortunately, as Burmester & Card point out, yolkless eggs may have chalazae. It has long been known that while in the uterus, the amount of the egg-white is doubled (Pearl & Curtis). The incoming fluid contains mainly inorganic ions, especially K' and HCO_3' (Scott, Hughes & Warren; Beadle, Conrad & Scott), but also (according to McNally[2] but not according to Hughes & Scott) some of the protein fraction known as ovoglobulin, which will be discussed further below (pp. 8, 671). After dissolving out a considerable amount of protein from the thick gel of the magnum, it becomes the outer thin egg-white. Its entry into the egg is slowed and eventually stopped by the deposition of the shell material. The composition of the proteins in the oviduct itself, as related to the secreted egg-white proteins, has been studied by Schenck, who obtained various more or less comparable protein fractions and believes that the percentage of amino-acids changes during the preparation of the proteins for secretion.

While the egg is in the uterus about 5 gm. of pure $CaCO_3$ are deposited on the shell membranes, first as knob-like calcite crystals, the *mammillae*, and then as the spongy layer, also composed of calcite but with much smaller crystals (Richardson; Stewart[1]). The calcium carbonate cannot be secreted in solution, but must appear as an extremely minute suspension (Beadle, Conrad & Scott). During laying, a pullet may be in negative calcium balance (Morgan & Mitchell), and even though this is later repaired, 10 % of the bone Ca'' of the adult hen is available for egg-shell formation (Deobald, Lease, Hart & Halpin).

It is interesting that hypocalcaemia and tetany are exceedingly rare in birds. An idiopathic case responded at once to parathyroid hormone given by Hutt & Boyd, who suggest that the rarity of the condition may be due to a special adaptive type of calcium metabolism in birds.

We come now to the contributions of histochemical and cytological type on oogenesis, many of which have come from the schools of Gatenby and of Marza. Unfortunately histochemistry has a long way to go before it can attain the certainty of direct chemical methods, and with the exception of certain special techniques, such as the Feulgen reaction, it is hard to know what to make of its results, especially when, as in some instances, they are expressed in a manner which seems to claim to be quantitative. It is hard, also, to find any common point between the cytological data and the results of chemical methods. The most hopeful line of advance here is undoubtedly the ultra-micro-chemical methods of the Copenhagen school. In the meantime all we can do is to refer to the best studies of histochemists and cytologists on oogenesis. A typical publication of the Irish school is the interesting account by Brambell of the maturation of the hen's egg, but the largest contributions on this subject are those of Marza (Marza & Marza; Marza & Golaescu; Marza & Chiosa; Marza, Marza & Chiosa; de Robertis; Grodziński). The deposition of fat in the fowl oocyte has been studied by Konopacka[2]. All this work and a very large further body of literature is efficiently summarised in Marza's monograph[4]. The exact study of the various types of vitelline globules has great importance, even from the point of view of experimental morphology (cf. p. 217), but they are so poorly characterised, and the methods available so relatively ineffective, that the subject is still in an early phase.* Other histochemical studies are available on the eggs of fishes (Marza[2], Marza & Guthrie; Konopacka[4]), cephalopods (Konopacki[3]), ascidians (Konopacki[4]), various worms and molluscs (Konopacki[1]), and crinoids (Chubb).

Recent additions to our knowledge of the maturation of the amphibian egg (reviewed in *CE*, p. 1681) have been those of Mestscherskaia, who estimated the respiratory rate of immature oocytes (Q_{O_2} -1 to -2), and Boyd[3], who studied rather completely the deposition of fats and sterols in the maturing oocytes. From her data a series of ratios could be calculated showing the progressive changes in the relative amounts of neutral fat, cholesterol (free or esterified) and phosphatides. Esterification of the cholesterol begins only at a relatively late stage.

One of the most classical observations on egg formation was Miescher's description of the exhaustion of the maternal body of the salmon during the spawning migration owing to the deposition of so much fat and protein in the eggs. This has lately been studied anew by modern methods by Davidson & Shostrom. Following this, Konopacka[3] has studied the appearance of the oil-globules which are so marked a character of fish eggs, and investigations have been made of the chemical changes in the blood during the spawning season in the salmon (Okamura[2]; Miyachi). During the maturation of teleostean eggs there are considerable changes in the fat-fraction (degree of saturation of the acids, length of chain, etc.), but investigators are not yet in full agreement concerning their nature (Channon & Saby; Lovern).

For insects, egg-number has been studied by Alpatov; Fauré-Fremiet & Garrault and Shapiro[1], in whose papers may be found a guide to this special literature. According to de Boissezon, mosquito eggs (*Culex pipiens*) come well to maturity even if the maternal organism has been prevented from biting, provided iron has been supplied to it in its larval stage.

I know of no chemical work on the eggs of mammals (see the book of Pincus), but analyses of the *liquor folliculi* have been made (Tafuri & Testa; Tafuri; Garufi). Marza[4] gives in his monograph an account of the action of hormones, especially the gonadotrophic hormone of the pituitary, in stimulating the growth of the oocyte. The papers of Pincus & Enzmann give access to the literature on these subjects.

1·2. Constitution of the eggs of birds

The fundamental data on which depend our knowledge of the constitution of the hen's egg and those of other species were given in *CE* (Pt. III, Sections 1·2–1·9). To *CE*, Table 2, should be added new analyses of the whole egg-contents (duck, Danilova & Nefedjova; turkey, Hepburn & Miraglia).

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* Lepeschinskaia makes the (inherently very unlikely) claim that in the eggs of birds some cells arise from certain of the vitelline globules without previous cell-division; her work has been severely criticised by Grodziński[5]. In other organisms, the role of wandering nuclei in the yolk may be indeed considerable (cf. p. 456).

1·21. The shell and shell-membranes

An extremely thin layer of protein, known as the cuticle, exists on the exterior of the shell; it has been described by Romankevitch and studied by Furreg and Marshall & Cruickshank. The last-named authors think that it assists the evaporation of water (see *CE*, p. 874) rather than the reverse, as it is continuous with protein material which fills up the pores in the shell (Almquist[1]). An average hen's egg-shell contains some 7000 of these pores, of mean diameter 0·04–0·05 mm., but the porosity varies with individual hens and is probably an inherited quality (Almquist & Holst). The pores are not more numerous over the air-space than elsewhere. The gas transmission through them may be measured by Romanov's method[6]. There are numerous studies of shell porosity (e.g. Rizzo; Dumansky & Strukova; Swenson & Mottern); it may vary considerably, through *mottled* (Holst, Almquist & Lorenz), where the water-content of the shell is uneven and causes uneven translucency, to *glassy* (Almquist & Burmester), where the number of pores is only half that of the normal egg-shell and the percentage protein in the shell twice the normal value. In such eggs daily water-loss is much reduced. Water-content of the shell is the most important factor in affecting light-transmission through it (Givens, Almquist & Stokstad). According to Almquist[2], the protein actually in the shell resembles collagen rather than keratin. Shell thickness is much the most important factor governing its breaking strength (Stewart[2]; Lund, Heiman & Wilhelm).

For the pigments in the shell, see below, p. 644.

The shell-membranes are generally thought to be composed of keratin fibres matted together. The latest analysis (Calvery[4]) indicates an exceptionally pure and typical keratin. Their structure has recently been re-examined by Szuman, and there is information to be had about their permeability properties (ions, water, membrane potentials, etc.) in the work of Yasumaru; Yasumaru & Sugiyama; Mizutani; Osborne, and Salvatori. Mucin fibres have been detected in them as well as keratin by Moran & Hale.

1·22. The white

The egg-white of the hen's egg (as stated in *CE*, pp. 265 ff.) consists of three distinct layers, an inner thin layer, a thick layer, and an outer thin layer. The first two of these accumulate in the oviduct, the last in the uterus. The layers differ considerably in the proteins which they contain (Hughes & Scott; McNally[1]), but former statements that the water-content is different cannot be substantiated (Holst & Almquist[1]). The great difference in viscosity must therefore be a colloidal phenomenon.

Table 1 (Hughes & Scott). *Egg-white proteins*

	Inner thin	Thick	Outer thin
Ovomucin %	1·10	5·11	1·91
"Ovoglobulin" %	9·59	5·59	3·66
Ovoalbumen %	89·29	89·19	94·43

It is to be noted that the fibrous ovomucin is present mostly in the thick layer, but according to Heringa & Valk keratin fibres are to be found there also. There is no difference in osmotic pressure between thick and thin egg-white, but the diffusion rate of water is quicker in the latter (Smith & Shepherd). The proportion of thick white is definitely an inherited character (Lorenz, Taylor & Almquist; S. S. Munro; Knox & Godfrey), but exactly what part it has to play during embryonic development is not yet known (see below, p. 620). On storage, or spontaneously in some eggs, the condition *watery white* is found (St John[2]; Holst & Almquist[2]); this has nothing to do with water-content of the layers, nor is the thick/thin ratio different, so that the change must be of a subtle colloidal nature. It does not affect the specific rotations of the proteins (Holst & Almquist[3]). It is probably one of the factors accounting for the fall in hatchability with storage time before incubation (see below, Fig. 1). Contrary to earlier statements (St John[1]) there is, according to the reliable measurements of Moran[2], little bound water in either kind of egg-white. Ovoalbumen binds no more than 0·26 gm./gm. protein.

In the egg-white there are four proteins the existence of which is well established, two albumens, ovoalbumen and conalbumen, and two glycoproteins, ovomucoid and ovomucin.* The fifth, ovoglobulin, still remains a matter of dispute, and may continue to do so for some time, since it is extremely difficult to prepare the fractions containing it free from ovoalbumen and ovomucin, and if it is present, it must be so in minute quantities. Conalbumen was shown by Hektoen & Cole[1] to be identical with serum albumen (later confirmed by Bruynoghe), and in the same paper they concluded that ovoglobulin also was an immunologically identifiable antigen. But they admitted that all their preparations of it contained ovomucin and ovoalbumen as well, and the sera which reacted against it reacted against these also. The "ovoglobulin" was not identifiable with serum globulin.† Later M. Sørensen, applying the orcinol method of Sørensen & Haugaard for sugar estimation to the egg-white proteins, accepted the existence of ovoglobulin, and regarded it as constituting 7 % of the total protein and as containing 4 %

Table 2. *Composition of egg-white proteins*

	Moisture and ash-free percentages					
	Ovomucin	"Mucin extract"	"Ovo-globulin"	Mother liquor	Ovo-albumen	Ovo-mucoid
Total N	12·6	14·3	14·3	14·3	14·4	12·6
Total S	1·8	0·8	1·1	0·9	1·5	1·4
Cystine	5·6	2·4	3·7	3·8	1·4	3·9
Glucosamine	10·4	3·1	6·4	4·5	1·6	9·7

N.B. "Mucin extract" is probably a mixture of occluded ovoalbumen and dispersed ovomucin; "mother liquor" is probably ovoalbumen contaminated with ovomucin.

In the terminology of K. Meyer's recent review, ovomucoid becomes α-ovomucoid and ovomucin β-ovomucoid.

Subsequent work on the immunology of egg-white proteins, especially with reference to species-differences, has been done by Hektoen & Cole[2] and Hooker & Boyd.

of mannose. Ovoglobulin preparations were found by Böhm & Signer to show flow birefringence (ovoalbumen does not; see below, p. 671), and by Wöhlisch & Belonoschkin to give a higher value in the Gans effect (depolarisation of the Tyndall beam) than any other protein tried. These results, though important, only show that mixtures of ovomucin with other proteins exhibit, as might be expected, strong evidence of anisometric particles.

In his careful separation of the egg-white proteins, E. G. Young[1] decided against the existence of ovoglobulin (see the figures given in Table 2). This is the most recent attack on the problem, but it is fair to say that other workers with special experience in protein chemistry, such as E. C. Bate-Smith, believe in the existence of ovoglobulin.

In E. G. Young's work, ovomucin accounted for 4·0 % of the total protein obtained, ovomucoid 1·4 % and ovoglobulin only 0·1 %. E. C. Bate-Smith[2] gives percentages as follows:

	% total protein
Ovoalbumen	77
Conalbumen	3
Ovomucoid	13
Ovomucin	7
Ovoglobulin	Traces

Young found that the chalazae consist mainly if not entirely of ovomucoid and ovomucin (cf. the work of Conrad & Phillips mentioned above).

Ovoalbumen has naturally been the subject of a large amount of work. Svedberg assesses its molecular weight at 34,500 when purified, but it may be much less in the intact egg, as sedimentation experiments on the undenatured egg-white indicate. Very accurate data are now available for its titration curve, either when undenatured (Loughlin) or denatured (Wu, Liu & Chou); its *p*H dissociation curve (Kekwick & Cannan); its particle size by ultra-filtration (Elford & Ferry); its amino-acid constitution (Vickery & Shore on the basic amino-acids; complete analysis by Calvery[2]) and its ultra-violet absorption spectrum (Marchlewski & Wierzuchowska). Its fragmentation under the action of enzymes such as papain, pepsin, polypeptidases, etc. has been intensively studied (Annetts; Calvery[5, 6], Calvery & Schock; Calvery, Block & Schock). Its carbohydrate group was said by Sørensen, on the basis of the orcinol method, to be mannose (1·7 % of the protein), but Neuberger, by a new procedure involving tryptic hydrolysis followed by acetylation and deacetylation under the mildest conditions, has obtained a polysaccharide which must be regarded as the prosthetic group. It consists of 2 molecules of glucosamine, 4 molecules of mannose, and 1 molecule of an unidentified nitrogenous constituent. It seems, however, that this carbohydrate group plays but an insignificant role in the immunological behaviour of ovoalbumen (Ferry & Levy).

In ovomucoid there is, of course, a much larger amount of carbohydrate than in ovoalbumen (e.g. 9·2 %, Sørensen; 10·4 %, Young). Zuckerkandl & Messiner-Klebermass regarded this prosthetic polysaccharide as an equimolecular mixture of glucosamine and mannose; Sørensen as 4 glucosamine + 3 mannose + 1 galactose. Masamune & Hoshino now claim it to be an equimolecular mixture of mannose and acetylglucosamine, and this is supported by the work of Iseki and of Fürth, Herrman & Scholl, who when hydrolysing ovomucoid with toluolsulphonic acid, obtained from this deacetylation 1½–2 mols. reducing sugar for every mol. of acetic acid formed. Mazza has contributed a complete study of the physical and chemical properties of ovomucoid.

In his review on the mucoprotein polysaccharides K. Meyer shows how close their relation is with the uronic polysaccharides of cartilage and umbilical cord. The mucopolysaccharide isolated by Levene & Lopez-Suarez from umbilical cord mucin has turned out to be a hyaluronic acid containing glycuronic acid and acetylglucosamine but no sulphur (Meyer, Palmer & Smith[1]; see also M. Suzuki), in which respect it differs from the chondroitin sulphuric acid of cartilage (cf. p. 443) which contains glycuronic acid and acetylchondrosamine (2-aminogalactose) as well as sulphur. Mucoitin sulphuric acid, like the latter except that acetylglucosamine occurs instead of acetylchondrosamine, is also known. None of these occur in egg-white, but their relation to the ovomucoids may be quite significant. Such polysaccharides form salts with the basic amino-acids of various proteins (Meyer, Palmer & Smith[2]) but may also occur in the dissociated form. Further research on the metabolism and morphogenetic significance of these compounds will be awaited with interest.

How far the various separable proteins of egg-white exist as such in the intact egg is a question not easy to answer. That they are to a large extent artefacts, formed during the application of the isolation methods, is believed by some (Block; Block, Darrow & Cary; Perov), and though their evidence, depending as it does rather largely on relatively small differences in amino-acid estimations, is not altogether convincing, the idea has attracted notable minds (e.g. W. B. Hardy, A. Kossel, etc.) and certainly cannot as yet be said to be disproved. Besides the discussions of Block, there is a review by Alcock on this subject, to which the reader is referred. On the other hand, ovoalbumen, formerly accepted as an entirely homogeneous protein, has been separated into two fractions by cataphoresis (Tiselius & Eriksson-Quensel; Longsworth, Lannau & McInnes). The number of proteins identifiable in egg-white by the acetone methods of Piettre was three, and Orrù[6, 8], in studying the changes in electrical conductivity of egg-white with temperature, found evidence for only two; but Young's[2] more recent work with the ultracentrifuge and the Tiselius apparatus suggests five or even six.

On the physical properties of egg-white there are numerous studies, e.g. electrical conductivity (Perov; Dolinov; Romanov & Grover); light transmission (Almquist, Givens & Klose), which depends primarily on the amount of ovomucin present; refractive index and its changes with storage (Romanov & Sullivan), etc. From Brooks & Pace we have an elaborate study of the CO_2-solubility in egg-white. The buffer value per gm. protein is $4{\cdot}8 \times 10^{-5}$ between pH 6·6 and 7·8; there is little or no carbamino-CO_2 present, and the solubility coefficient is a little higher than that calculated, probably owing to the presence of traces of lipoids. The egg-white contains 4·5 milliequivalents of organic acids (Hosoi); on development this falls but later rises.

Sulphur compounds in egg-white exist only in the proteins, the sulphur-content of which can be entirely accounted for as cystine and methionine (Marlow & King; Toennies).

In later sections we shall consider the part played during development by the constituents of the white, but it may be mentioned here that according to G. Schmidt[2] the development of the embryo on the yolk *in vitro* will only go on if both the proteins and the dialysable substances of the white are present around it. Isotonic salt solutions will not do.

1·23. The vitelline membrane and its physiology

The vitelline membrane of the hen's egg is composed of a network of keratin fibres about 25 μ thick. It is of particular interest because it separates two systems of widely different properties, and upon their efficient separation the possibility of later normal embryonic development to some extent depends. On one side of the vitelline membrane there is a thick and viscous material containing approximately 50 % water, and on the other a much less viscous phase of which as much as 85 % may be water. The yolk solid is about two-thirds fat and one-third protein; the solid of the white is almost entirely protein. The yolk has double the percentage of ash of the white. Corresponding to these many other differences there is a great difference in osmotic pressure (about 1·5 atmospheres). Not merely is the total osmotic concentration different on the two sides of the membrane, but also there is practically no agreement between the constituent items in that concentration. Thus there is much more potassium, sodium, and chlorine in the yolk than in the white, but the difference is not the same in each case. The osmotic pressure difference is equivalent to about 2 kg. sq. cm., but it is obvious that the membrane is not submitted to any such mechanical pressure. The membrane must be permeable to water, since the yolk swells and osmotic equilibrium is attained in the intact egg if it is stored for some weeks; moreover, yolks readily take up water from dilute solutions (Smith & Shepherd; Orrù[1]). Permeability to some salts, though less pronounced, must exist, for Orrù[4] found that water in which yolks were immersed showed a gain in electrical conductivity, and an increased depression of freezing-point, and reached a *p*H of 5·7. Orrù[5] also brought forward evidence indicating the passage of glucose, sucrose and raffinose through the membrane into intact yolks. Hevesy, Levi & Rebbe have shown that although heavy water quickly distributes itself between white and yolk when injected, radioactive sodium phosphate does not, but remains for long in the white.

Straub & Hoogerduyn rightly considered that the Schreinemaker equations were inapplicable, and the distribution of ions in yolk and white is so different from what it would be on the Donnan equilibrium theory that the latter must be regarded as not alone sufficient to account for the case of the vitelline membrane of birds' eggs. Indeed, the potential difference between yolk and white, measured by Capraro & Fornaroli, is only 2–3 mV. rather than the 130 mV. required by such equilibria.

As pointed out by Needham & Smith, there are other remarkable facts about the separation of yolk and white. Yolk contains both vitamins B_2 and B_1; white only B_2 (Chick, Copping & Roscoe). Like all the other differences this difference will persist for many weeks in a stored egg, but in time equilibration occurs. Again, at first all the zinc is in the white and all the copper in the yolk (L. K. Wolff), but after a long period of storage this unequal distribution disappears. The *p*H differs greatly, the yolk is acid, the white alkaline.* The principal facts are summarised in the following table:

Table 3. *Properties of avian yolk and white*

	Yolk	Egg-white	Blood
Δ°, freezing-point depression	−0·55	−0·44	−0·64
Osmotic pressure, atmospheres	6·6	5·3	7·7
% water	50	85	—
Fat % dry weight	66	0	—
Protein % dry weight	33	100	—
Ash % wet weight	1·5	0·7	—
Glucose % wet weight	0·27	0·55	—
*p*H	4·5–6·0	8·6–9·6	—
Milliequivalents/100 gm. water:			
K′	5·81	3·73	
Na′	9·30	2·61	
Cl′	7·89	5·15	

Straub & Hoogerduyn made two important experiments. On putting yolk into a parchment capsule with egg-white outside, osmotic equilibrium was attained in 24 hr. instead of many weeks, as in the intact egg. This was subsequently confirmed by Needham[5]. But Straub & Hoogerduyn also placed the yolk of a fresh egg in diluted egg-white, and after leaving it there for several hours, replaced it in ordinary white, whereupon its osmotic pressure, which had been lowered, rose above, not merely to the same level as, that of the ordinary white. There was thus a recovery of the natural hypertony of the yolk. They concluded that the "living" vitelline membrane tended to encourage the exit of water from the yolk or to impede its entry, or to encourage the entry of salts and impede their exit. This conception fitted in well with the views on the performance of thermodynamic work at living membranes, which were then becoming current (1931; respiratory rate and osmotic work, etc.), and the view of the vitelline membrane as such a membrane was widely adopted, as by Kluyver and A. V. Hill[2]. Hill[1] himself contributed the observation that the osmotic pressure equilibration curves in unincubated eggs (as measured by the vapour-pressure method) were alike whether the eggs had been kept aerobically or anaerobically. If energy were needed, therefore, it must come from some source not involving oxidation in free oxygen.

This led to a series of papers in which the situation was much clarified.† Smith & Shepherd showed that the fundamental experiment of recovery of hypertony by the yolk was to be explained simply on the basis of a temporary heterogeneity of the contents of the vitelline membrane; when the yolk is placed in diluted egg-white or water, water enters, forming blisters of very low osmotic pressure, and not being incorporated in the yolk owing to its great resistance to diffusion.* The recovery of hypertony is therefore an illusion, for the main mass of the yolk has never been diluted at all. Straub later himself adopted this explanation. Stored at 25°, complete osmotic equilibrium between yolk and white is reached after about 70 days; between 0 and 25° the temperature coefficient of this equilibration is between 1·5 and 2. This was later confirmed by Orrù[3]. The shape of the curves and the regular effect of temperature strongly indicate that the primary process is diffusion, hindered by various complex factors. The viability of stored fertile eggs shows a quite different relation to temperature, since there is an optimum at 8–10° and a slow decline of viability on either side of this point (Moran[1]; see Fig. 2). The converse of the Straub-Hoogerduyn experiment was also performed by Smith & Shepherd, namely the concentration of the egg-white by rapid evaporation, but there was no perceptible effort on the part of the yolk to maintain its hypertony.

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* This is also true, as Imamura[1] and others have shown, for reptilian eggs such as those of marine turtles. During development the yolk becomes more alkaline and the white more acid (Schkljar; Rubinstein; Gaggermeier; Sharp & Powell; Baird & Prentice), confirming *CE*, p. 857.

† Summarised in Needham, Smith, Shepherd, Stephenson & Needham.

In contrast to earlier statements in the literature, the infertile unincubated hen's egg gives off no respiratory CO_2 and consumes no O_2 (M. Smith[1]). There is a certain liberation of CO_2 from fresh eggs, but it falls off rapidly to minimal values, and undoubtedly arises from the action of traces of acid substances on the calcium carbonate of the shell. The vitelline membrane itself exhibits no dehydrogenase activity and neither the membrane nor the yolk show any measurable respiration *in vitro* (Needham, Stephenson & Needham). Bacteriologically sterile yolk produces minute traces of lactic acid and of a substance or substances estimatable as ethyl alcohol; the larger amounts described in the older literature were certainly due to bacterial contamination. It is very unlikely that the energy so produced is available for work at the vitelline membrane.

As stated above, yolk and white, separated only by a parchment or collodion membrane, rapidly come to osmotic equilibrium. An apparatus was devised by Needham[5] for investigating the osmotic properties of the isolated vitelline membrane, and it was found that when placed between two salt solutions of different strengths, it opposes no resistance to rapid equilibration. There is thus a paradox which is still not fully resolved. With yolk and white on either side, equilibration is slower than with salt solutions, though not nearly so slow as in the intact egg. It is probable that the physical structure of the yolk and white has some feature which retards the attainment of equilibrium, and which is not wholly destroyed by the mixing of each phase on its removal from the egg. The slow attainment of equilibrium in the intact egg is probably due to the co-operation of the two phases with the membrane. It must be remembered that the yolk is laid down layer upon layer, not poured into the vitelline membrane, and its lipoidal molecules or lipoprotein complexes are almost certainly highly oriented. This was the view of Hardy (in Hale & Hardy), who emphasised the hindrances to free diffusion in the yolk. Moreover, structures hindering rapid diffusion of water in the white were found by Smith & Shepherd.

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* Baldes later believed that the layers of yolk nearest the vitelline membrane have normally a lower osmotic pressure than the central core, so that a continuous gradient would exist from the interior of the yolk to the shell, but this could not be confirmed by Moran & Hale.

The actual existence of a difference in osmotic pressure between yolk and white has been denied by some authors, but mistakenly. Grollman[2], the first of these, used dialysis and the thermo-electric technique for freezing-point determination, but Meyerhof[1], in whose laboratory the work had been done, later showed that his results were vitiated by the absence of a correction for the water initially in the collodion membranes. Bateman[1] and Baldes also vindicated the use of the thermo-electric technique as applied to viscous liquids such as yolk. Howard[2] reinvestigated the question with the Beckmann thermometer and again obtained the same osmotic pressure for yolk and white, but these results were decisively rejected by M. Smith[2], who showed that they were due to plateaus of temperature without the presence of ice in the mixture, caused by a temporary balance between the heat gained by stirring and lost to the cooling bath. Ice forms much more slowly in viscous fatty media than in aqueous solutions, but in a crucial experiment, it formed and persisted at $-0{\cdot}58^\circ$ but disappeared at $-0{\cdot}53^\circ$. Similar conclusions were reached by Johlin[1], by Hale & Hardy, and by Weinstein, so that there can be no doubt of the osmotic pressure difference between yolk and white. This can be demonstrated very simply, as Johlin[2] showed, by placing yolk and white in an apparatus such that water can distil over from the more dilute to the more concentrated phase; in such circumstances yolk always gains at the expense of white.

When yolk and white are mixed together, or with solutions of various salts, their physical behaviour is quite anomalous. There occurs on simple mixing a removal of osmotically active substances (Bateman[1]) and some precipitation (Baldes). The changes of viscosity when yolk is diluted with solutions of salts at different *p*H (Zawadzki), and the changes of electrical conductivity with temperature (Orrù[6]), are very complex.

In the vitelline membrane itself there is said to be some mucin as well as the keratin (Moran & Hale). By the aid of the micro-manipulator three layers can be detected in it, an outer thin layer, the thick middle layer, and an inner thin layer. The thin layers stain as mucin, the main one as keratin. The average bursting strength of the vitelline membrane at laying is about 4500 dynes/sq. cm. (Moran[3]); on storage the membrane weakens but becomes more elastic. As the egg ages water-intake by the yolk when transferred to water becomes more rapid (Orrù[1]). After 40 hr. incubation of a fertile egg the membrane becomes so weak over the germinal area that its bursting strength can no longer be measured. With the growth of the yolk-sac it disappears. Moran[3] recalls in this connection the hatching enzymes of lower vertebrates and invertebrates (see p. 627), but though there may be an evolutionary connection, such an enzymic mechanism can hardly be of any value to the chick embryo. Trypsin rapidly weakens the vitelline membrane, which is interesting in view of the existence of a protease with tryptic properties in the thick egg-white (Balls & Swenson).

1·24. The yolk

The yolk of the hen's egg consists of concentric layers of white and yellow yolk, with a central core of white yolk, which can often be seen in eggs coagulated by boiling. The details of the structure were described in *CE*, p. 235. No new analyses of white and yellow yolk have been made since those previously described (*CE*, p. 286), but further analyses of the white yolk, the "erste Nährung" of the embryo, would be very desirable. Sometimes the peripheral white yolk layer is laid down unevenly, producing a characteristic spotty appearance of the yolk (Schaible, Davidson & Moore).

Egg-yolk proteins have been the subject of an excellent review (up to 1932) by Jukes & Kay[3]. The principal protein of the yolk is the phosphoprotein vitellin. Its amino-acid distribution has been investigated with modern methods by Calvery & White, who obtain higher values for tyrosine, arginine and lysine than previous workers. The most reliable figure for phosphorus-content is 0·92 %. The figure of Calvery & White for tryptophane is somewhat lower than that of Tillmans & Alt. Methionine in vitellin has been estimated by various authors (Baernstein; Scharpenack & Jerjomin); there is about 2 %.

Study of the hydrolysis breakdown products of vitellin has thrown much light on how the phosphorus is combined in the phosphoprotein molecule. Posternak & Posternak were the first to identify serine-phosphoric acid (phosphoserine) as the principal mode of attachment, and this was later fully established by Lipmann & Levene and Levene & Schormuller. Posternak & Posternak obtained an "ovotyrin"* from vitellin containing 10–14 % phosphorus and 28 % *l*-serine.† Such phosphoproteoses seem to occur to a small extent in normal yolk, and it was found that after allowing yolk to autolyse a much increased yield could be obtained. On decomposition the ovotyrin of the Posternaks gave a good deal of pyruvic acid, probably because of the formation of aminoacrylic acid after removal of the phosphoric acid groups from a phosphoserine chain, and subsequent oxidation. The natural phosphoproteoses are rather easily hydrolysed by alkali (heating for 1 min. with *N*/NaOH), an instability which the Posternaks suggested might be of some biological importance in phosphorus transfer from raw materials to embryonic nuclein and bone phosphate (see *CE*, p. 1202).

Measurements of the reaction constant for hydrolysis of vitellin by Lipmann[2] showed it to be identical with that for phosphoserine itself, and Rappoport, using a new method of estimation for serine, concluded that all the P of vitellin is combined as phosphoserine (in contradistinction to casein, where one-third of the P is not in phosphoserine). Agreement has not, however, been finally reached. Blackwood & Wishart noted that pepsin liberates much acid-soluble N and not much acid-soluble P from vitellin, while trypsin does both; they concluded that there must be two kinds of P complex in the molecule, one highly resistant to enzymic attack. Herd obtained phosphopeptones of varying N/P ratios, suggesting that the P is unequally distributed through the molecule. In pepsin there is an accumulation of those fragments which contain much P and so are resistant to this enzyme. Herd's results would perhaps not be incompatible with the conception of localised concentrations of phosphoserine chains.

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* Many phosphoproteoses have been isolated from the fragmentation of vitellin, differing slightly in their content of P, Fe, serine, etc., and the names "ovotyrin", "ichthiotyrin", "vitellinic acid", "paranuclein" and "haematogen" are practically synonymous.

† They obtained it also from the vitellin of fish eggs, hence the name "ichthiotyrin". The early workers on vitellin called vitellin "ichthulin" if it came from fish eggs, "batrachiolin" or "ranovin" if it came from amphibian eggs, etc., but these names are quite unnecessary and should be given up. The prefix "ovo-", to vitellin is also obviously superfluous.

The ultra-violet absorption spectrum of vitellin is given by Marchlewski & Wierzuchowska.

The second protein of egg-yolk is livetin (*CE*, p. 294), a pseudo-globulin containing only 0·067 % P. It occurs to the extent of about 20 % of the total yolk-protein. Its amino-acid distribution has been studied with modern methods by Jukes[1], Jukes & Kay[1], and Kay & Marshall. It contains about the same amount of methionine as vitellin (Baernstein). Jukes & Kay[2] have shown immunologically that in all probability livetin is identical with serum globulin (cf. the identity of conalbumen with serum albumen). Physical constants of livetin will be found in Kay & Marshall.

Earlier work on protein-bound carbohydrate in the egg had made it very likely that the yolk as well as the white contained a good deal of prosthetic polysaccharide of the mannose-glucosamine type, and Jukes found 4 % in livetin by the Tillmans-Philippi method. It was left, however, for Onoe[1] to isolate from the yolk a third protein, vitellomucoid, analogous with the ovomucoid of egg-white, and containing 10·1% carbohydrate. The work of Orrù[6,8] on the effect of temperature on the electric conductivity of yolk* had already indicated the existence of at least three proteins, and Piettre, by his acetone method, believed he could detect four. Onoe's vitellomucoid proved to have only mannose and glucosamine in its prosthetic group.

Whether these proteins are at any time preferentially absorbed by the embryo we do not know (but see below, p. 620, for some information of this kind on vitellomucoid).

The richness of egg-yolk in neutral fats, phosphatides and sterols has already been pointed out (*CE*, pp. 294, 1218). An egg of average weight (55 gm.) has in its yolk 5·58 gm. neutral fat and 1·28 gm. phosphatides, of which 0·68 gm. is lecithin (Lintzel; Bornmann). The structure of the phosphatides has been closely examined. Rae found that in yolk lecithin the β-form of glycerophosphoric acid predominates (i.e. the fatty acids are arranged symmetrically to the choline-phosphate radicle), in contrast to brain lecithin, in which the α-form predominates, and liver lecithin, which is an equal mixture of the two. There is agreement (Sueyoshi; Sueyoshi & Furukobo; Yokoyama; Riemenschneider, Ellis & Titus; Spadola & Riemenschneider) that the fatty acids of yolk lecithin are mainly isopalmitic, oleic, linoleic, clupanodonic, and 9:10-hexadecenoic, with very little stearic and palmitic.† According to Yokoyama, five varieties of the molecule are

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* Data also in Romanov & Grover.

† The following data are appended:

Acid	No. of C atoms	No. of double bonds
Palmitic	16	0
9:10-Hexadecenoic	16	1
Stearic	18	0
Oleic	18	1
Linoleic (Linolic)	18	2
Linolenic	18	3
Clupanodonic	18	8
Arachidonic	20	8

present: dioleic-α-lecithin, di-*iso*palmitic-α-lecithin; oleic-clupanodonic-α-lecithin, oleic-*iso*palmitic-β-lecithin, and dioleic-β-lecithin. In egg-yolk kephalin, on the other hand, Nishimoto found the following varieties: palmitic-stearic-α-kephalin, palmitic-arachidonic-α-kephalin, palmitic-stearic-β-kephalin, palmitic-arachidonic-β-kephalin, and diarachidonic-β-kephalin. The possible significance of these highly unsaturated acids as raw material for embryonic tissues, which may not themselves be capable of desaturating fatty acids, will not escape notice.

Kay believes that the presence of free glycerophosphoric acid in fresh yolk is doubtful; in the fresh egg there is nothing which can act as substrate for phosphatase. But on storage the lipases are active and the acid-soluble phosphorus of the yolk increases (Pine); eventually the inorganic phosphorus of the white also (Janke & Jirak).

The egg-yolk of the hen, and many other birds, contains 1·75 % cholesterol (Miyamori); but that of the duck about twice as much (Gaujoux & Krijanowski; Achard, Levy & Georgiakakis).

Yolk dissolves a good deal more CO_2, according to Brooks & Pace, than white, because of its higher lipoid content. The yolk contains 5·0 milliequivalents of organic acids (Hosoi); on development this rises slightly and later falls.

There is no spermine in eggs (Dudley & Rosenheim). Details on pigments and enzymes will be found in the appropriate sections (see below, pp. 70, 652). Vitamins will also be discussed later (pp. 23, 616), but their presence in yolk and white may be summarised with a select bibliography as follows:

Table 4. *Vitamins in the avian egg*

	Yolk	White	
A	+	–	Russell & Taylor; Cruickshank & Moore
B_1	+	–	Baker & Wright; Plimmer, Raymond & Lowndes
B_2	+	+	Chick, Copping & Roscoe; Aykroyd & Roscoe
P-P	+	–	Goldberger, Wheeler, Lillie & Rogers
C	–	–	Hauge & Carrick; Ray
D	+	–	Guerrant, Kohler, Hunter & Murphy
E	+	–	Card, Mitchell & Hamilton

1·25. Inorganic constituents

New complete analyses of the ash of yolk and white have been made by Straub & Donck (they are in close agreement with those given in *CE*, Table 27) and Yosida has made estimations of total basic ions in yolk and white. These workers were only concerned with elements present in considerable quantity, but there exist studies with modern spectrographic methods which give some information about the rarer elements also.* According to Drea, ash from the whole hen's egg contains Al, Ba, B, Ca, Cu, Fe, Mg, Mn, P, K, Ru, Si, Na, Sr, Ti and V.† Of these, strontium and vanadium appear to be concentrated by the maternal organism in the egg, as the egg contains more than the diet. Boron and manganese occur only in the yolk. Apparently absent were F, Ag, Cr, Pb and

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* See below, p. 651, for the results of application of similar methods to foetal tissues.

† It is interesting to compare this list with the trace elements found in newborn mammals and their meconium. Work of Ramage; Sheldon, Ramage & Ramage; and Rusoff & Gaddum shows that these include Al, Ba, Cu, Mn, Sr, Sn, Zn, and sometimes Pb and Ag, but not Sb, Be, Bi, B, Cd, Co, Ln, Th, Ti, V, Yt, or Zr.

Mo. Similar work by Cazzaniga demonstrated Fe, Na, K, Al and Ca in both yolk and white, but Mg only in the white. Love adduces evidence of a heavy metal, probably lead, but this was in Australia, where, as work previously described (*CE*, p. 305) has shown, eggs laid by hens in the vicinity of lead mines may contain considerable amounts of lead (see also p. 556), deposited in the yolk as lead oleate.*

The halogen elements in the embryonic raw material have received a good deal of attention. In an elaborate paper on the chloride of yolk and white Mankin[2] concluded that the white Cl′ is usually slightly higher than the yolk Cl′. This is at variance with the results of Straub & Donck, but for this ion the differences between yolk and white are not very great. All investigators are agreed that the iodine content of the egg, though initially very small (about 0·1 mgm./kg.), can be raised many hundreds of times by adding such substances as KI, kelp, etc. to the diet (Almquist & Givens; Wilder, Bethke & Record; Berkesy & Gönczi; Scharrer & Schropp; Jaschik & Kieselbach), though the increase in the eggs laid is not directly proportional to the increase in iodine-content of the diet. Zaitschek found that added iodine raised the hatchability of the eggs 13 % and the number of eggs laid by 12 %, but no such effect was observable by Johnson, Pilkey & Edson. By injecting sodium bromide into laying hens Purjesz, Berkesy & Gönczi were able to increase the bromine of the eggs from 23γ to 7 mgm./egg largely in the yolk. By injecting sodium fluoride into laying hens the fluorine of the eggs could be raised from zero to 40γ/egg. This was confirmed by Phillips, Halpin & Hart, who on adding fluorine-containing mineral supplements to the diet found that the fluorine was deposited in the yolk in ether-soluble combination with lipoid material (like lead). Fluorine in the yolk rose from 0·3 mgm./100 gm. to 1·46. Fluorine toxicosis inhibited laying without reducing the size of the eggs.

By feeding colloidal lead orthophosphate to the laying hen W. B. S. Bishop[4] was able to double the amount of lead in the eggs (0·047 mgm./egg to 0·09 mgm./egg); even in doses subtoxic for the adult the hatchability was much reduced.† Selenium will also go into hen's eggs, in quantities sufficient to be dangerous (Franke & Tully; Franke, Moxon, Poley & Tully; Moxon & Poley). Molybdenum has been recognised qualitatively in egg ash (Mankin[1]). In agreement with L. K. Wolff (see above, p. 12), Guérithault found that copper occurs in the yolk to the extent of 1·8 mgm./kg. (wet wt.), but there is none in the white. Manganese also occurs only in the yolk, where there is about 2·5 mgm./kg. (dry wt.), as estimated by Skinner & Peterson, Peterson & Skinner, and McHargue. Finally, silicon has been studied by King, Stantial & Dolan, who find it to be present in both the yolk and white in very small amounts.

By feeding diets of very different ionic composition Schkljar[3] claims to have brought about considerable changes from the normal alkali reserve of both yolk and white.

Doubtless the most important problem which arises with regard to the inorganic constitution of the hen's egg is the state in which iron is found there. In the earlier literature (cf. *CE*, p. 291) it was often noted that various phosphoproteose preparations, obtained from the breakdown of the vitellin molecule, contained high percentages of iron. This was the reason for the introduction of Bunge's term "Haematogen". The still unsolved riddle of the origin of haemoglobin in the embryo makes a decision as to the state of the iron in the egg of some importance. The modern phase opened when R. Hill[1] in 1930 applied the α-α′-dipyridyl method of iron estimation to egg-yolk. Haematin iron is protected from this reagent, but on adding a reducing agent to egg-yolk in its presence, a deep red colour immediately develops, suggesting that the iron is present as colloidal ferric hydroxide. Since the amount of iron as estimated directly by the dipyridyl method was equal to that estimated after ashing, Hill concluded that the yolk contained no iron in strongly bound organic combination such as haematin. These results were later confirmed by Lenti.

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* This work was criticised on technical grounds by H. B. Taylor, but the reply on behalf of the original group of workers by W. B. S. Bishop[5] was convincing.

† See p. 556.

This was widely thought to imply the liquidation of Bunge's haematogen, but McFarlane continued to uphold the view that iron exists in weakly bound organic form for the following reason. After alkaline hydrolysis of lecitho-vitellin (0·25 N/NaOH) a fraction was obtained, not precipitable with trichloracetic acid but precipitable with neutral lead acetate, containing all the iron and 25 % of the copper of the original material. The metals were still present in the material (which was presumably a phosphoproteose, though no phosphorus determinations seem to have been made) after removal of the lead with H_2S (Fe, 1·23 %; Cu, 0·0082 %), and there was no reaction with potassium thiocyanate or sodium diethyldithiocarbamate. McFarlane thought that iron, more loosely bound in vitellin than in haematin, might react with dipyridyl, but the point was left untested. Contributory suggestions that yolk does indeed contain organic as well as inorganic iron arose from two sources, the histochemical work of Marza, Marza & Chiosa, and the fact, established by Elvejhem, Hart & Sherman, that yolk iron is only poorly available for haemoglobin regeneration in rats, its value in this respect, unlike that of most other sources, not corresponding to the dipyridyl assay. Histochemical methods are, however, much too uncertain to decide a question of this nature, and it was subsequently shown that the regeneration discrepancy is due to the sulphur of the egg which, combining in the gut with the copper to form insoluble CuS, withholds from the animal the copper necessary for the catalysis of haemoglobin synthesis (Sherman, Elvejhem & Hart; Rose, Vahlteich & McLeod). The discrepancy disappears if copper is added to the yolk given.

McFarlane's views are not supported by the most recent work of Tompsett using thiolacetic (thioglycollic) acid. In trichloracetic filtrates of yolk there is nothing that interferes with this method of estimation, yet such filtrates show no iron and iron alum added to yolk before the precipitation completely disappears. But if the reaction with thiolacetic acid is carried out before precipitation with trichloracetic acid, then the iron present is readily estimated in the filtrate, and not only does this quantity of iron agree exactly with that found after ashing, but ferric iron added to fresh egg-yolk may be quantitatively recovered. It may therefore be regarded as fairly clearly established that the iron of the yolk is in inorganic form adsorbed in some way on to proteins or lipoprotein complexes. As ordinarily prepared, the "Fe-content" of vitellin is in the neighbourhood of 0·045 % (McFarlane).

McFarlane also believes that copper is organically bound in vitellin (about 0·003 %).

1·3. The effect of pre-developmental factors on development

That the development of the chick embryo is affected by factors operating on and in the egg before development has started admits of no doubt. These factors manifest themselves as differences of hatchability, a subject on which we owe most of our information to American investigations.* The chemical composition of the egg itself can be affected by dietary or genetic influences, and the fate of the egg after laying can be affected by several environmental variables (cf. the reviews by Landauer[23] and by Jukes & Kay[1]).

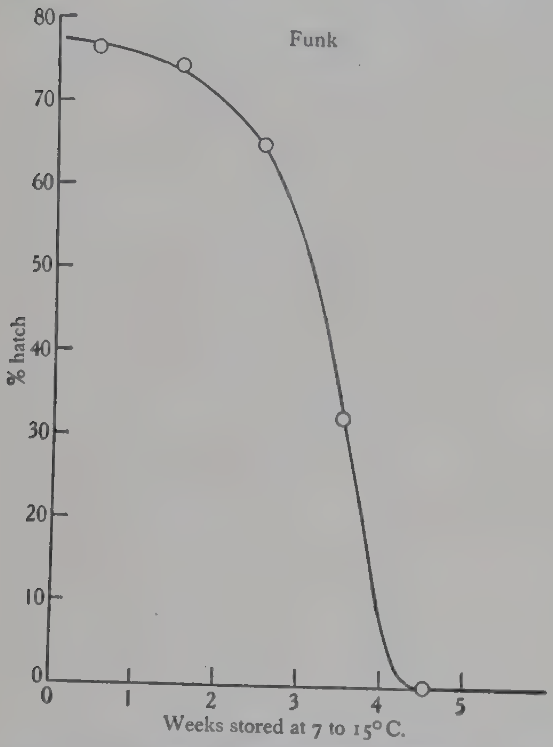

Fig. 1. Decline of hatchability with time of storage before incubation (hen's eggs).

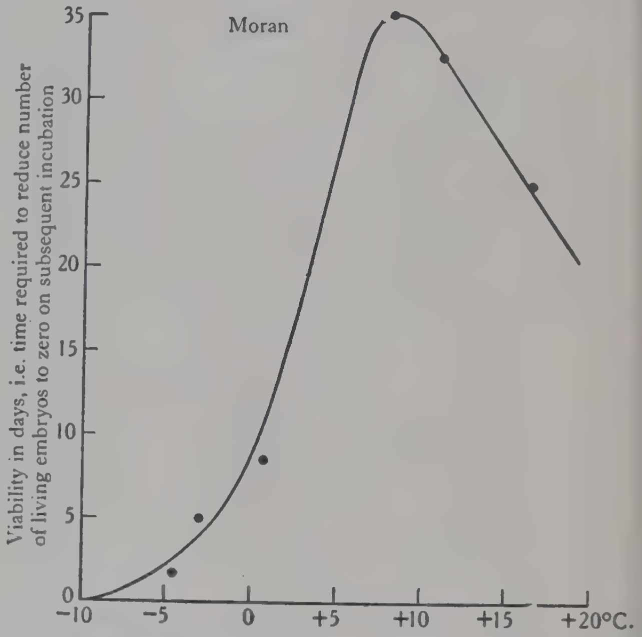

Fig. 2. Optimum temperature for storage of fertile hen's eggs.

The accompanying curve (Fig. 1) taken from Funk's statistical work, confirmed by Kaufman[8] and many others, shows how hatchability falls off with time between laying and the beginning of incubation. As Fig. 2 shows, Moran[1] found an optimum temperature for such storage. The exact interpretation of these findings is not entirely clear, but the unphysiological equilibration of the yolk and white is probably involved.† Hays & Nicolaides believe that eggs laid containing advanced gastrulae have a high probability of successful hatching, while those laid as early pre-gastrulae have a low probability.

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* Card[3] has made a useful bibliography of the publications of U.S. Agricultural Experiment Stations on subjects of importance for embryology.

† Loss of water by evaporation is not in itself harmful up to a certain point. Kaufman[8] found that after 5 weeks, when hatchability is reduced to zero, only about 2% water had been lost from the eggs, yet when the water-content of fresh eggs had been reduced by 5% by exposing them to low atmospheric pressures, hatchability is unaffected. Moreover, hatchability could not be restored by soaking the kept eggs before incubating them. The margin of safety for water-loss is therefore, in the hen's egg, appreciable (but see pp. 36, 64).

Once development has started, the optimum temperature lies between 38·9 and 39·4° C. for natural incubation, and between 37·2 and 37·8° C. for forced-draught incubators with uniform heat distribution. Optimum humidity is also an important variable; it lies between 50 and 60 %. There is a considerable literature on the details of these questions because of their industrial importance; it may be approached through the reviews of Landauer[23] and of Romanov[10], and the paper of Byerly[6].

The effect of egg size on hatchability has been subject to some dispute, but it is now generally accepted that hatchability falls off at both extremes of variation from the normal, more especially so at the higher egg-weights (Godfrey; Warren; Funk; Axelsson; Shibata & Murata), cf. Fig. 3. According to Penionskevitch[4] variations of egg-weight are due largely to variations in the total weight of the egg-white. It is interesting, therefore, to find that such characters as percentage and physical properties (particularly gel rigidity) of the thick egg-white are inherited (van Wagenen & Hall; van Wagenen, Hall & Wilgus; Hall & van Wagenen; Lorenz, Taylor & Almquist), and that hatchability could be correlated with the properties of the thick egg-white. If it is non-rigid or present in too small amount the embryos die between the 2nd and the 7th day of incubation; if there is too much of it, or of the rest of the egg-white, they die at later stages. Abnormally large and heavy eggs have an abnormally long incubation period (Byerly[5]; McNally & Byerly), and such eggs contain at 48 hr. incubation embryos with more somites than normal. In abnormally small eggs, however, development is not correspondingly retarded. McNally & Byerly found that there is an optimal hatchability in eggs containing 20-somite embryos at 48 hr. incubation.

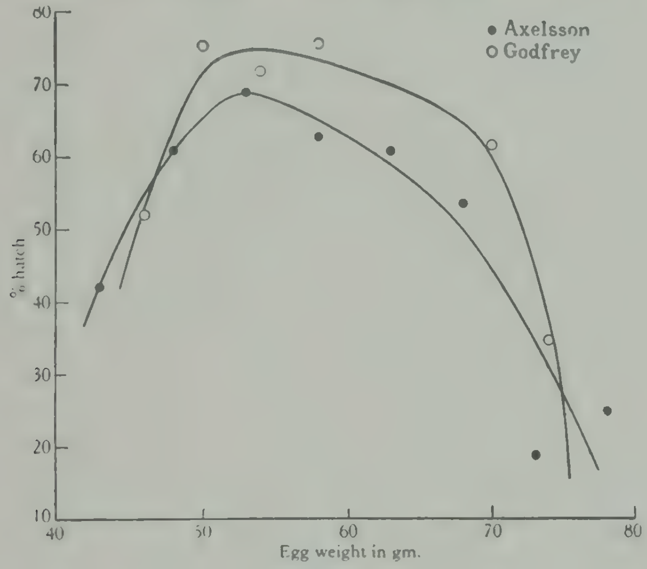

Fig. 3. Relation of egg weight and hatchability.

The characteristics of the egg-shell are also important for hatchability. Excessive porosity will lead to excessive evaporation of water and desiccation of the embryo (Axelsson). Shell porosity is affected by both autosomal and sex-linked genetic factors, but may be artificially produced (as a phenocopy, see p. 392) by withholding calcium from the diet of the laying hen, as in the experiments of Buckner, Martin & Peter. Penionskevitch[5] points out that the shell-membranes are also probably important for hatchability, since they may vary in their capacity for holding water in case of undue desiccation, and hence in their liability to develop a parchment-like condition which makes hatching impossible.

At present there is little evidence that yolk qualities play a role in hatchability. Percentage solids (Bronkhorst & Hall[2]) and the shape index, probably a measure of vitelline membrane elasticity (Hall & van Wagenen), are unrelated to it. There may be some connection between the pigmentation of the yolk and hatchability; this will be referred to below in connection with pigments (pp. 23, 653). Penionskevitch[4] thinks that "efficiency of yolk utilisation" may be an important factor.

A good deal of work has been done on the effects of diets of different protein composition upon the eggs and the embryos arising from them. In general the results are difficult to interpret. If the composition of the egg-proteins is affected by the protein in the diet, it must be in relatively subtle ways which do not clearly reveal themselves to the still relatively crude technique at our disposal for protein characterisation. Pollard & Carr fed pigeons on different single seed grains and reported that the egg-proteins, as studied by van Slyke analyses, varied considerably. High hatchability was correlated with high melanin (hence probably high tryptophane) content. Continuation of the work later however by Gerber & Carr gave much less striking results, in that the differences in mono-amino and di-amino fractions were smaller, though contributory immunological evidence was adduced to show that perceptible differences existed between egg-proteins of different dietary origin. It was also claimed that embryos from eggs of different dietary origin showed differences in growth rate, though no such differences appeared later in the work of Penquite & Thompson on embryos from eggs produced by hens on high and low protein diets. The experiments of Titus and his colleagues indicate that any differences must be very small. Calvery & Titus found no significant differences in egg-proteins of different dietary origin, except that the amide-nitrogen of all proteins was slightly lower on a soy bean diet. Titus, Byerly & Ellis could alter the percentage dry matter in the yolk and the percentage protein in the yolk solids by adjusting the diet, but this was not correlated with hatchability. Byerly, Titus & Ellis found that on certain diets, especially those largely composed of maize, wheat, soy bean, fish meal, etc., there was a significantly high mortality of the embryos during the 2nd week of incubation. This is not the time at which either of the two normal mortality peaks occur (see p. 64). Whether this was due to a deficiency of essential amino-acids or of vitamins was not elucidated, though all the necessary vitamins were believed to be present in sufficient amount. The largest investigation was that of McFarlane, Fulmer & Jukes, who obtained wide variations in hatchability, and in the stage of development at which the maximal mortality occurred, by employing diets containing buttermilk powder, fish meal, tankage, meat meal, etc. Thus with tankage there was a 50 % mortality in the first mortality period (3rd–5th day), while with cod-liver meal the mortality rose to 50 % in the second mortality period (18th day onwards). Yet no significant difference was found in the composition of the protein from low hatchability eggs as regards total nitrogen, total amino-nitrogen, tyrosine, tryptophane and cystine content. There were, indeed, variations in total ash and iron-content of the proteins, but they bore no relation to the hatchability. One striking effect of tankage was noticeable, namely an anaemia of the early stages (4th day), haemoglobin failing to form in the blood islands, and an almost colourless fluid circulating through the heart. For this phenomenon no explanation was found. It was certainly not due to iron or copper deficiency. It does not follow that all samples of tankage show these effects, since high hatchabilities were reported with it by Francisco, Chan & Fronda.

Variations may also be induced in the lipoidal fractions of hens' eggs. Cruickshank has established that unsaturated fatty acids, present in unsaturated oils in the feed, are deposited in the yolk, but that on the contrary an abnormally high proportion of saturated fatty acids in the diet does not affect the normal mixture of saturated and unsaturated fatty acids in the yolk. This is supported by Lorenz, Almquist & Hendry, who obtained positive Halphen tests, indicative of an unsaturated fatty acid, on yolks formed by hens which had eaten malvaceous plants or their seeds (e.g. cotton-seed meal or mallow seed). Almquist, Lorenz & Burmester then found that if, after the depôt fat of a laying hen shows a strongly positive Halphen test, all the eggs larger than $\frac{1}{4}$ in. diameter are removed from the ovary, the yolk subsequently deposited on the remaining eggs will show no positive Halphen test, though the depôt fat will continue to show it. And this is still the case even on a fat-free diet. This ingenious experiment is in agreement with a similarly planned one carried out by Henderson & Wilcke using the dye Sudan III, and it goes far to show that depôt fat is not necessarily used by the hen in laying down the fat of the yolk, a synthesis from carbohydrate and protein being sometimes preferred. On the other hand, as is well known, substances giving unusual flavours and odours do easily become incorporated in eggs (cf. McCammon, Pittman & Wilhelm).

Cruickshank & Moore find that the vitamin A content of egg-yolks (first separated from accompanying carotenoid yolk-pigments by Gillam & Heilbron in 1935) can be raised five times by enriching the diet of the laying hen with it; but as in the case of iodine and other substances, the rate of increase in the egg is far from being directly proportional to the increase in the diet. It is stated by Barancheev and Kirsanov that there is a relation between yolk-colour and hatchability, the darkest yolked eggs developing best, and since the lipochromes are by far the most important coloured substances in yolk (Bisbey, Appleby, Weis & Cover) it may be that lipochromes other than vitamin A have a physiological significance. Deficient vitamin A in eggs, as is generally acknowledged since the work of Holmes, Doolittle & Moore, will produce low hatchability, and up to a certain point the restoration of hatchability will go on in proportion to the vitamin added to the diet of the laying hen (Sherwood & Fraps; Payne & Hughes; R. M. Smith; Bearse & Miller).

It is doubtful whether vitamin B_1 plays any role in hatchability (Ellis, Miller, Titus & Byerly). Vitamin B_2, however, is essential, and, like B_1, can be varied in the egg by feeding different diets (Ellis, Miller, Titus & Byerly; Bethke, Record & Wilder; Lepkovsky, Taylor, Jukes & Almquist; Engel, Phillips & Halpin). As already mentioned (p. 17), egg-white contains only B_2 while yolk contains both vitamins. Norris, Wilgus, Ringrose, Heiman & Heuser have shown that, up to a certain concentration of vitamin B_2 in the diet, there is a direct proportionality between hatchability and concentration, though after the optimal point is reached further additions give no further improvement (see also the work of Ogorodny & Penionskevitch, referred to on p. 389).

The presence of vitamin D in the egg is essential for successful development. It varies greatly in amount with different diets. The question of whether sterol provitamins can be formed by the irradiation of eggs with sunlight or ultra-violet light, discussed in *CE*, p. 1360, is still not settled, but it seems probable that the penetration of radiant energy through the shell is insufficient to do more than compensate for slight dietary deficiencies (Landauer[9]; Martin, Erikson & Insko; Martin & Insko; Insko & Lyons[2]; Graham, Smith & McFarlane; Payne & Hughes; Murphy, Hunter & Knandel; Carver, Robertson, Brazie, Johnson & St John; Guerrant, Kohler, Hunter & Murphy; Russell & Taylor). By a laborious process

Windaus & Stange separated the provitamin from egg-yolk cholesterol and found it to be ergosterol, present at 0·18% in the crude material. Schoenheimer & Dam and Menschick & Page had already shown that ergosterol, added to the diet of laying hens, is deposited in the eggs produced (for structural formulae, see p. 251). The mechanism of embryonic avitaminosis-D has been more closely examined by Insko & Lyons[2], who found that embryos in such eggs fall more and more behind the normal controls in their calcium and phosphorus content as development progresses. The peak of mortality occurred on the 18th day. As Landauer[15] showed, the presence of the *creeper* gene (see p. 382) causes an earlier onset and a severer expression of D-deficiency symptoms, so that the two mechanisms may be regarded as additive.

Excessive vitamin D in the diet will cause a decline of hatchability even to zero (Branion & Smith), but the effect on the embryos is still very obscure.

It is generally held that vitamin E is essential for successful development. This is difficult to prove, however, for no diets for fowls have so far been devised which do not probably involve other deficiencies also, or cause other disturbances. As we shall see later, interesting specific morphological abnormalities arise in E-deficient embryos (Adamstone[1], cf. below, p. 264), and there are several reports that hatchability is seriously affected by E-deficient diets (Card; Card, Mitchell & Hamilton; Barbas; Barnum; Dols).

Other accessory food factors of hitherto unknown nature may be involved in normal development, as Nestler, Byerly, Ellis & Titus observed low hatchability on diets not deficient in any of the known vitamins.

We come lastly to the more subtle effects on hatchability arising from the condition of the parent birds. From the work of Axelsson and others it may now be considered as established that the hatchability of eggs laid during the first or pullet year is slightly better than that of eggs laid during the second year, and the work of Hyre & Hall strongly suggests that this decrease in hatchability continues during the subsequent years. Somewhat contrary to expectation is the fact that hatchability varies positively with current egg production but not with antecedent egg production; hens able to lay eggs at a high rate on a particular diet will, in general, produce a higher percentage of hatchable eggs than those only able to lay at a low rate on the same diet (Byerly, Titus & Ellis). Under certain conditions (*frizzle* gene, Landauer; sudden falls in external temperature, Funk; individually characteristic low blood-sugar, Bronkhorst & Hall[1]) the laying hen may apparently be unable to deposit all the necessary substances in the eggs, and falls in hatchability may ensue.

1·4. Constitution of the eggs of lower vertebrates and invertebrates

1·41. Reptiles and Amphibia

We have a good deal of information about the composition and metabolic changes during development of the chelonia (especially the turtle *Thalassochelys corticata*; see *CE*, pp. 898 and 1043, etc.), but concerning the composition of saurian eggs extremely little is known. Studies of the egg-white of the loggerhead turtle, *Caretta* (Kondo; Kondo, Yamada & Nagashima), show that it contains two proteins provisionally classified as globulins, and an albumen. This differs from hen's egg-albumen, however, in coagulating 10° higher, containing more tryptophane and tyrosine (spectrophotometrically deduced) and having a different isoelectric point (*p*H 5·75 instead of 4·89). The egg contains 50% egg-white, 43% yolk and 7% shell. Analytical data for the percentage composition and proportion of the amino-acids histidine, arginine, lysine, tryptophane, tyrosine and cystine in turtle vitellin are given by Kusui[2]; during development there was no change in the composition of the protein. Recently Fukuda[2,3] has given fairly complete analyses of the eggs of a dozen species of snakes; in general, as would be expected, their composition is similar to those of birds, but they seem to have much less carbohydrate, both free and combined.

In view of the overwhelming importance of the amphibian embryo in experimental embryology, it is very surprising that no studies of its chemical constitution with modern methods have been made. Pierantoni has given an account of the yolk-platelets of amphibian eggs, but there is here an important gap which needs filling. Most of the newer information not contained in *CE* concerns the egg-jelly, composed of fairly pure mucoprotein. Long ago Schulz & Ditthorn and v. Ekenstein & Blanksma isolated galactose from frog egg-jelly mucoprotein, and the recent work of Schulz & Becker has shown, chemically and by fermentation methods, that the prosthetic polysaccharide consists of galactose and glucosamine (cf. p. 9). It is generally known that the jelly is soluble in strong cyanide solutions, which, remarkably enough, do not irreversibly interfere with the subsequent normal development of the embryos, but it is not so well known that exposure to ultra-violet light will also dissolve the egg-jelly (Sypniewski). It has often been supposed (e.g. *CE*, p. 323) that the jelly furnishes nourishment to the emerged tadpoles, which may be seen clinging to it with their suckers, but a special attempt to decide this question (Savage[1]) gives no grounds for such a belief. The mean nitrogen-content of young tadpoles fell in exactly the same way, owing to consumption of yolk, whether they had been allowed to remain on the jellies or not. Disappearance of the jellies is due to bacterial action, not consumption by the tadpoles. It was noted, nevertheless, that the differentiation of the gut seemed to be more advanced in the tadpoles from the jellies than in those from batches where the jellies had been taken away; this contrasts with the statement of Szepsenwol[3] that removal of axolotl tadpoles from the jellies results in an acceleration of neural differentiation owing to the earlier onset of external stimuli. In a later paper, Savage[2] estimated the total carbohydrate in tadpoles from batches with and without jelly, and was able to show that no significant difference exists. The nutritive function of the jelly can therefore probably now be ruled out, but Savage suggests that it may act as a heat-insulator and thermostatic device.

The tension at the surface of a salamander egg (*Triturus virescens*) has been determined by Harvey & Fankhauser.

1·42. Fishes

As regards the eggs of teleostean fishes there is little to add to our former account (*CE*, pp. 331, etc.) except that Mörner has shown that the *dl*-cystine, said to have been isolated from herring eggs by Steudel & Takahashi, was in all probability taurine. Bernheim & Bernheim describe the phosphoprotein of the eggs of the trout *Cynoscion nebulosus*, which, when undenatured, contains free-

–SH groups like myosin or haemoglobin. Konopacka[3] has given an account of the formation of the oil-globule so conspicuous in teleostean eggs, in *Smaris alcedo*; and the surface tension at the interface between such globules and the yolk has been measured by Harvey & Shapiro for *Scomber*, *Phycis* and *Merliccius*.* To account for its remarkably low value it is considered necessary to assume some unknown substance in the yolk of high surface activity, identified in the case of the mackerel as a globulin by Danielli & Harvey.

The protein of the "chorionic" membrane of salmon eggs (*Salmo salar*) was analysed by Young & Inman, who describe it as a pseudo-keratin of mesodermal origin. The permeability properties of the egg-membranes of the trout have been described in an interesting paper by Gray[3], having in mind the work already discussed (p. 12) on the properties of the vitelline membrane of the hen's egg.† The normal trout egg, like those of all organisms developing in fresh water, is impermeable both to water and intracellular electrolytes, the barrier being the (inner) vitelline, not the (outer) chorionic membrane. Later work by Krogh & Ussing, using heavy water, and P. Adler, using dyes, strikingly illustrated this. Injuries to the egg-cell cause exosmosis and a coagulation of the vitellin which spreads rapidly all over the egg from the point of injury. Osmotic stability varies with age, mechanical disturbances, Ca″ concentration of the medium, and the nature and concentration of the exosmotic agent; owing to the impermeability of the membrane no work is required to maintain the normal concentration of salts and water within.‡ Precipitation of the vitellin within the egg can also be brought about by galvanic currents, according to Scheminsky & Scheminsky, anodically if weak, cathodically if strong.

Teleostean eggs contain vitamin A (McWalter & Drummond) and B_1 and B_2 (Lunde, Kringstad & Olsen).

Analytical work on the eggs of selachian fishes shows that they possess less fatty material than would be expected from their highly lecithic nature. Ray eggs (*Raia oxyrhyncus*) studied by Schmidt-Nielsen & Stene and Schmidt-Nielsen, Aas, Astad & Leonardsen gave the following figures:

	% Wet weight			
	Dry weight	Ash	Protein	Fat
Egg-white	4·0	2·7	1·4	0·0
Yolk	36·0	1·3	28·0	6·7

* And by Harvey & Schoepfle for the oil-globule naturally occurring in daphnid eggs (see also Chambers & Kopać).

† We do not know whether the "chorionic" membrane of the teleost egg is to be regarded as analogous with the shell-membrane or the vitelline membrane of the hen's egg. If it is analogous with the shell-membrane the perivitelline space represents the white. If it is analogous with the vitelline membrane of the hen's egg we might expect to find some protoplasmic layer inside around the hen's egg yolk corresponding with the protoplasmic vitelline membrane of the fish, but for this there is no evidence.

‡ Other fish eggs also have highly impermeable membranes, e.g. *Oryzias* (Ikeda[2,3]), but in this case the embryos within are quite sensitive to external ionic disequilibria. But the marine teleosts have eggs with much more permeable membranes, according to Krogh, Krogh & Wernstedt. The crustacean *Artemia* (the brine-shrimp) is a curious case; the adults live in brines of 25% salinity or more, and show a remarkable constancy of their internal medium (Medvedeva; Warren, Kuenen & Baas-Becking). But the eggs and nauplii need lower osmotic pressures and are sensitive to abnormal ion balances (Boone & Baas-Becking; Jacobi & Baas-Becking; Baas-Becking, Karstens & Kanner; Matthias). Those who wish to pursue these questions further are referred to the monograph of Krogh on osmotic regulation in aquatic animals.

Δ for white was −2·08°, for yolk −2·90°. The yolk contained 5·89 % urea (see *CE*, p. 343). The eggs of the shark, *Heptranchias deani*, studied by Ono[1], have more fat:

	%
Water	47·10
Fat	23·30
Protein	25·13
Ash	0·81

Ono[2] made a special study of the physico-chemical properties of the fatty fractions in this case. Of the neutral fat 21 % was solid acids (isopalmitic, palmitic, stearic) and 79 % liquid (oleic, linolenic and iwashic). Unsaponifiable material (12 % of the crude fat) included octadecyl and cetyl alcohols as well as the highly unsaturated selachyl alcohol, besides cholesterol (see also below, p. 250). An interesting study of the yolk-proteins of the ray (*Raia batis*) by Fauré-Fremiet[3] confirms the earlier discovery of a livetin in elasmobranch eggs (*CE*, p. 334); in this case it forms about 10 % of the total protein. The lecitho-vitellin complex occurs in the form of strongly birefringent rectangular or pyramidal platelets containing 17 % phosphatide and 1·4 % cholesterol.

Among numerous observational studies on elasmobranch egg-cases that of Aiyar & Nalini on the Indian *Chiloscyllium griseum* may be mentioned, but the only physico-chemical work on them is that of Fauré-Fremiet[7] and his collaborators on the keratin, which they undertook in connection with their researches on protein microfibrils in general (see p. 676). The manner of secretion of this protein in rays is described by Garrault & Filhol. Fauré-Fremiet & Baudouy[2] showed that prokeratin, obtained by Fauré-Fremiet & Garrault from the nidamentary gland of *Raia*, *Scyllium*, *Mustelus*, etc., differs from the protein as found in the completed egg in containing somewhat more sulphur and somewhat less nitrogen. The completed egg-case shows birefringence and a highly oriented micellar constitution (Fauré-Fremiet[7]; Filhol & Garrault). Imbibition properties of selachian egg-case keratin were studied by Baudouy.

We shall have a good deal to say later on, under the heading of the embryo and the environment, about the eggs of selachian fishes.

1·43. Arthropods

Apart from data on the constitution of insect eggs, which will be mentioned incidentally to other questions later in this book (pp. 36, 455), there is little to be said here. At the beginning of development, the egg of the grasshopper, *Melanoplus differentialis* (cf. pp. 578 ff. for a fuller discussion of this embryo), contains 17–20 % dry wt. fatty acids, and this amount is reduced by half during development (Slifer[1]). In a later study, Slifer[4] examined the fatty acids from a number of orthopteran eggs. Iodine numbers varied from 128 in the case of *Dissosteira carolina* to 166 in the case of *Arphia sulphurea*, and melting-points from 25·5 to 39·5° in another series. As the females of all the species were kept at constant temperatures during the egg-laying period, the differences, though possibly due to environmental adaptations, cannot be due to the direct effect of different environmental temperature. In spite of the similarity of iodine values, the fats differ widely in the proportion of solid and liquid fatty acids, and probably in chain length. Sacharov thinks that there is a relation between cold-hardiness of insects and total fat-content of their eggs, but a larger set of comparative figures than those offered by him will be needed to establish this. From the unsaponifiable fraction of silkworm eggs Ongaro has isolated a saturated hydrocarbon $C_{28}H_{58}$ which recalls the hydrocarbons found in fish oils (p. 250).

Remarkable changes in the viscosity of the yolky silkworm egg may be followed, according to Tirelli[1], by normal development. After heating to 50° for ½ hr. the viscosity of the press-juice is 50 % greater than normal, and this change is irreversible, for it persists for two months. Yet there is no interference with normal development.

The inorganic constitution of insect eggs has been little studied, but Melvin has shown that certain insects, such as *Blatta*, accumulate copper, in this case up to 18 mgm./kg. dry wt. in the eggs.

Gause[1] has shown that egg size in *Drosophila* is affected by the maternal diet in larval life.

1·44. Echinoderms

Much the most outstanding contribution in this section is the list of physical and chemical constants for the sea-urchin egg (*Arbacia punctulata*) prepared by E. N. Harvey[4] in 1932. This gives data for all dimensions, densities, viscosity, surface tension, osmotic properties, *p*H, chemical constitution, rate of development, etc. Unfortunately there is little since that date with which to supplement it.* Costello[1], however, has ascertained the relative volumes occupied by the formed components of twenty-six species of marine eggs (of other groups as well as echinoderms), using a centrifuge method. This volume varies from 40 % in the egg of *Hydroides* to 82 % in that of *Polychoerus*, and in every case it exceeds the volume of the osmotic dead space. This is in agreement with all other authors, such as Lucke[1], who studied the osmotic behaviour of centrifuged *Arbacia* egg fragments and found that it closely paralleled that of the whole egg (though the osmotically inert material was unequally distributed) and Białaszewicz[1]. It is not quite clear whether the "dispersed phase" of Białaszewicz is identical with the osmotic dead space of the American authors. In *Echinometra lucunter*, for example, this osmotically inert volume is 34 % of the initial egg volume (Leitch). A large body of literature exists on the permeability properties of echinoderm eggs, but since most of it is occupied primarily with biophysical problems and so far has thrown little light on morphogenesis, it will not be reviewed here. For further details the reader is referred to the review of Lucke & McCutcheon.

Bank has described the properties of the jelly surrounding the echinoderm egg. He has also made the curious observation that a stratification of the egg constituents, analogous to that produced by centrifuging, can be obtained if the eggs are placed in a 1 % solution of caffein. The phenomena must be similar, for it was noticed that batches of eggs which were unusually resistant to centrifugal stratification were also resistant to stratification by caffein.

Work on the cytology of maturation of the egg of *Echinometra lucunter* (Tennent, Gardiner & Smith; Miller & Smith) was followed by some chemical work by D. E. Smith. Contrary to earlier work on echinoderm eggs (*CE*, p. 1049) a good deal of polysaccharide was found in this one (12·7 % dry wt.). The total solids amounted to 28 % wet wt.; cholesterol was present to the extent of 0·6 % wet wt. and phosphatides 1·8 % wet wt. There was less neutral fat than glycogen (6·3 % dry wt.), so that proteins would account for 80 % dry wt., a striking example of the richness in protein in marine alecithic eggs commented on in *CE*, p. 313. The iodine value of the fat from *Arbacia* eggs was studied by Payne, who found that it varied with the temperature of the water in which the maturation and spawning had taken place, from 98 at 0° to 150 at 20°. The higher figure is in agreement with one given by Terroine, Hatterer & Roehrig. Navez, who thinks that a fatty acid derivative of echinochrome (see p. 215) may exist, also found evidence of highly unsaturated fatty acids and possibly of squalene (see p. 250).

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* Ballentine[2] gives accurate figures for the nitrogen content of *Arbacia* eggs.

A short communication by O. Glaser[5], overlooked in former publications, indicates the existence of considerable amounts of copper in echinoderm eggs; this should be further investigated.

1·45. Molluscs and worms

The most peculiar characteristic of molluscan eggs is the presence of relatively large amounts of a polysaccharide formed from *d*-galactose, not glucose. This galactogen has been studied in a number of papers by May and May & Kordovich. The eggs of the snail, *Helix pomatia*, contain no less than 37·8 % of it (dry wt.) —6·02 % wet wt.—and no glycogen. It has a sinistral rotation $\alpha_D - 22{\cdot}73^\circ$ and gives no iodine reaction. Both galactogen and glycogen accumulate in the snail's body for hibernation, but only the former is deposited in the eggs. Apart from the gonad, the slime gland is the only other organ which contains galactogen, but as the total egg material has more galactogen than what is present in these organs, it is probable that glycogen from other organs can be converted into it. In starvation the reverse process takes place. Slug eggs also contain galactogen (36·2 % dry wt.), but May and May & Weinbrenner were also able to isolate traces of the polysaccharide from other sources such as the gonads of *Anodonta*, the eggs of the carp (where it makes up 1·2 % of the total polysaccharide, itself only 0·2 % dry wt.), the rabbit ovary (where 7 % of the total polysaccharide is galactogen), and even the human placenta (where 1 % of the total polysaccharide is galactogen). May suggests that galactogen may be, in the higher animals, the mother substance of the milk-lactose galactose and the brain-cerebroside galactose. Progress towards elucidating the exact chemical constitution of galactogen is recorded by Baldwin & Bell. Snail eggs also contain a curious laevorotatory tetrasaccharide (4-*d*-galactosido-*l*-glucosan) according to May & Stadelmann.

The X-ray investigations of F. K. Mayer have yielded the fact that in the shells of embryo gastropods, the calcium carbonate is laid down, first in the form of vaterite, then aragonite, and finally calcite. Anomalies in gastropod eggs are described by Löhner and Cardot.

The Japanese gastropod, *Hemifusus tuba*, has been the subject of further researches. Figures for the composition of its vitellin and mucoid are given by Kumon, and the proportion of various amino-acids in the egg-capsule protein has been investigated by Sagara, who takes it to be a keratin. Ankel, on the other hand, finds chitin in the egg-capsules of the European *Nucella* (*Purpura*) *lapillus*. These fragmentary data will acquire greater interest when we know more of the chemical constitution of gastropod eggs. During development, according to Kumon, the total nitrogen of the egg interior rises, but he could not find a corresponding fall in the total nitrogen of the egg-capsules (see below, p. 626). Lastly, the eggs of the land-snail, *Achatina fulica*, have recently been analysed by S. Fukuda[3]. These appeared to contain very little fat—only 0·1 % wet wt.—but besides the protein (10·2 %) an equivalent amount of something else which was neither protein nor fat (11·4 %), probably the galactogen of May. There was also, according to Fukuda, an unusually large amount of silicate, but this should perhaps be accepted with caution, as the egg-mass might have been imperfectly freed from earth.

It seems that the eggs of lamellibranch molluscs have fatty acids with the highest iodine values yet recorded for eggs (Terroine, Hatterer & Roehrig). A table given by these authors follows:

Eggs	Iodine value of fatty acids
Hen	103
Frog	142
Sea-urchin	135
Pike	163
Herring	208
Lamellibranch mollusc (*Pecten*)	232

Manganese has been noted as a constant constituent of the eggs of a number of fresh-water lamellibranchs (Bradley; 0·63 % dry wt.).

The first satisfactory analyses of the eggs of cestode worms have been recorded by Smorodintzev, Pavlova & Ivanova.

	% Water	% Organic substance	% Ash	% Protein	% Fat
Diphyllobothrium latum	72·6	26·59	1·15	21·64	4·95
Taeniarhynchus saginatus	77·19	22·30	0·66	15·42	6·88

The low fat-content is worth attention. The adult worms have, of course, a much higher water-content.

1·5. The inheritance of immunity

Among the chemical substances present in eggs antitoxins and antibodies must be included (cf. *CE*, pp. 1444 ff.). While in birds (Ramon; Jukes, Fraser & Orr; Fraser, Jukes, Branion & Halpern) and in reptiles (Grasset & Zoutendyk) both antigens and antibodies are deposited in the eggs, in mammals the placenta is permeable only to antibodies and not to the antigens themselves.* This subject has been well reviewed by Grasset, who gives an account of the literature. It has long been known that at early developmental stages, the power to react to a foreign antigen by the production of active immunity is absent or present only to a slight degree. Thus Freund using rabbits and Kliger & Olitzki using guinea-pigs, in *ad hoc* experiments, found young animals to be poor antibody producers (Jaffé). According to Gebauer-Fuelnegg the formation of antibodies by the chick embryo proceeds only very weakly, and Grasset was quite unable to obtain any antibody formation when diphtheria toxin, either in active or inactive (anatoxin) form, was injected into chick embryos at any time during incubation. The anatoxin did not interfere with normal development, but the toxin quickly killed the embryos. Weinberg & Guelin had exactly the same experience with *B. sporogenes*; the chick embryo can make no agglutinin for it.

In general, therefore, it may be said that embryonic and new-born animals may possess passive immunity, caused either by the deposition of antibodies in the egg or by their passage through the placenta.† During post-natal development this passive immunity disappears and is replaced by the active immunity characteristic of adult life, i.e. by the power to form antibodies in response to antigens entering the blood circulation. This general rule may be summarised in the following diagram, from Grasset (Fig. 4).

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* This is constantly being confirmed, see e.g. the paper of Tooney on the *B. coli* agglutinin titre of maternal and foetal blood in man.

† Note that this condition resembles that of adult invertebrates, judging from the useful review of Huff. They depend on natural, rather than acquired, immunity.

It is to be noted that embryonic passive immunity falls off before the rise of adult active immunity, so that there should be, as is actually observed, a period of low level of immunity in early post-natal life. The inability of the embryonic and newly born or hatched organism to form antibodies is particularly interesting in view of the lack of species-specificity as between tissues, which embryos of different species or even groups and classes show (see p. 348). Rodolfo has done an admirable piece of work on the permeability of the rabbit placenta to antibodies. This placenta changes in construction during embryonic development. On the 10th day the two bloods are separated by three membranes, the foetal endothelium, the foetal mesoderm and the foetal ectoderm; on the 22nd day there are only the first two of these; and on the 29th day only the foetal endothelium. The placenta is thus of the haemo-chorial type at the beginning (see p. 81) but haemo-endothelial at the end. Correspondingly, Rodolfo found that the ratio of titres foetal blood/maternal blood rose from 0·2 at 23 days to 0·8 at 30 days in the case of the agglutinin to *B. abortus* and from 0·05 at 23 days to 1·0 at 30 days in the case of the haemolysin for sheep blood corpuscles.

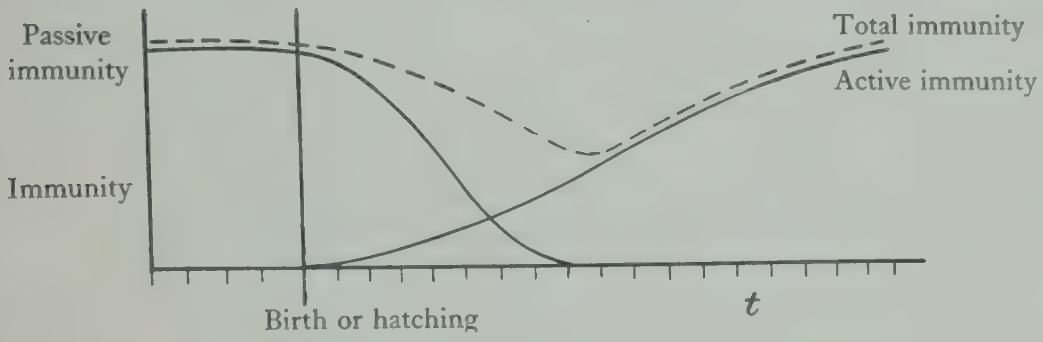

Fig. 4. Embryonic origins of immunity.

In this connection the origin of the human blood groups is of interest (Haselhorst; Keeler & Castle; Abe; Kaboth). Oku has shown that they originate in the foetal circulation about the 2nd month of pregnancy, the iso-antibodies coming from the maternal circulation. Keeler & Castle think that small amounts of the iso-agglutinogens also come through the placental barrier, but are immediately destroyed in the foetal blood. A difference between the blood group of maternal organism and foetus is not believed to have any deleterious influence upon the latter, though some authors, such as Garrasi, believe that optimum intra-uterine conditions, as manifested by birth weight and placenta weight, are present when the blood groups are the same.

It may be added that the technical feat of raising chicks from the egg bacteria-free has been accomplished by Balzam.

1·6. The egg and the environment

1·61. The problem of protection; larval compression and ovoviviparity

In what follows we shall have to review a rather complex interplay of factors involved in the relation between the developing embryo and the environment, including various aspects of metabolism and nutrition. But it is all ultimately connected with the devices employed by animal organisms to give their offspring the best chances of survival. The most primitive type of development is probably that in which a large number of eggs with relatively little yolk are laid. Although this has the advantage of facilitating the dispersion of the species, the destruction of a large proportion of the embryos before or after they hatch entails a severe strain on the parent organisms. Moreover, the existence of minute free-swimming larvae in the plankton is a positive bar to the colonisation of fluviatile fresh-water. On balance, therefore, it proved more satisfactory to increase the size of the eggs and to prolong the developmental period so that the embryo, when it hatched, could face the hostile world with much enhanced probability of survival. This meant compressing larval stages into the embryonic period. The whole range of egg sizes, from that of the sea-urchin to that of the ostrich, is an illustration of this tendency.

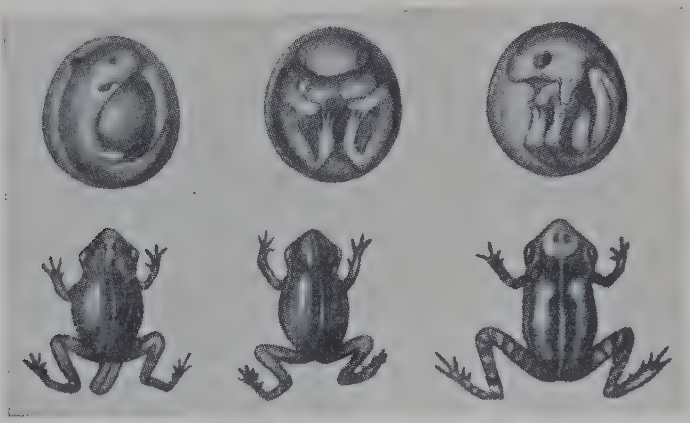

Fig. 5. Developmental stages of the tree-frog *Hylodes martinicensis*.

A glance at Brinley's table of the egg sizes of fishes is instructive in this connection, for Rass was able to conclude that (among the teleosts at any rate) those species with the largest eggs and the longest developmental periods had the widest geographical distribution. But the larger the eggs became, the more valuable they were to the species, and hence we see arising all kinds of arrangements for the care of the developing embryos and larvae (cf. the reviews of Wunder). High degree of parental care, small number of eggs, large egg size, and long developmental time, all go together, as do the converse phenomena. Thus fishes, marine or fresh-water, which show parental care, lay from 80 to 200 eggs in a season; those which do not, lay from 30,000 to 700,000. For amphibia, parallel figures are 4–270 as against 1000–28,000. Excellent cases of larval compression are found in groups as widely separated as the amphibia—Fig. 5, taken from Wunder, shows the later stages of the development of the tree-frog *Hylodes martinicensis*, in which the tadpole stage is passed entirely within the egg—and the nemertines (Schmidt & Jankovskaia).*

In the course of time it was found that the protection of the eggs could best be assured, not merely by the continued presence of the parent organisms, but by their actually carrying the eggs about with them. This is what happens to-day in the case of the toad *Alytes obstetricans*. But such close contact led inevitably to the complete retention of the eggs within the parent organism, either at some level in the genital tract (e.g. the ovary or the uterus) or in some specially prepared brood-pouch on the ventral or dorsal surface, or even in the mouth, either of the male or female. In some such way ovoviviparity arose. And the process did not stop there, for the maternal organism could carry the offspring for almost unlimited periods if means were taken to reduce the structures interposed between the foetal and maternal blood circulations. So at last true viviparity was attained, as in the mammals.

One of the most elaborate investigations of the origins of ovoviviparity is that of Berrill[2] on ascidians.† The ovoviviparous Molgulids go through a course of development in which the tailed larva is suppressed, and osmotic hatching takes the place of the hatching enzyme (cf. p. 627). For further details the original papers must be consulted.

1·62. The evolution of the cleidoic egg

We now come to the degree of dependence upon the environment shown by the eggs of different organisms. It has for long been recognised (more particularly in *CE*, Sections 6·6, 9·15) that some animals have eggs much more completely closed off from their environment than others, and the word *Cleidoic* (closed box) has come into use to designate the former. A typical non-cleidoic egg would be that of a cephalopod, which is not supplied by the maternal organism with sufficient ash or sufficient water to make one complete embryo, but has to absorb many of its constituents from the sea. The mammalian egg must also be regarded as non-cleidoic, for even its organic constituents are not stored in it at the beginning of development, but have to be provided later on from the maternal organism. A typical cleidoic egg, on the other hand, would be that of a bird, where all that the embryo requires, except the oxygen, is provided within the closed system. Between these extremes many intermediate grades are found (see Table 5).

The most fundamental need of the non-cleidoic egg is for water. This was fully discussed on a previous occasion (*CE*, pp. 889 ff.), but some newer observations justify further comments here. The clearest cases are those of two fresh-water organisms, the trout (*Salmo*) and the axolotl (*Amblystoma punctatum*), for which equations can be drawn up, as in Table 6, showing the amount of

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* Cf. also the discussion on p. 76. High degree of parental care without large egg size or viviparity can also be attained if the life-span is so adjusted that the earlier generations of offspring can assist in protecting the later ones. In some such way, as Wheeler has taught, arose the social habit among insects.

† See also Child[2].

Table 5. *Evolution of the cleidoic egg*

	Solid		Liquid	Gas	
	Organic substances (proteins, carbohydrates, fats, lipoids, sterols, etc.)	Ash	Water	Oxygen	
OVIPAROUS:					
Aquatic					
Echinoderm (no yolk), e.g. sea-urchin (*Echinus*)	+	−	−	−	NON-CLEIDOIC
Ctenophore (yolk), e.g. (*Beroe*)	+	−	−	−	
Cephalopod, e.g. squid (*Sepia*)	+	−	−	−	
Crustacean, e.g. sand-crab (*Emerita*)	+	−	−	−	
Selachian fish, e.g. dogfish (*Scyllium*)	+	−	−	−	
Teleostean fish, e.g. trout (*Salmo*)	+	+	−	−	
Intermediate					
Amphibian, e.g. frog (*Rana*)	+	+	−	−	
Chelonian reptile, e.g. turtle (*Thalassochelys*)	+	+	−	−	
Terrestrial					
Land amphibian, e.g. tree frog (*Hyla*)	+	+	+	−	CLEIDOIC
Saurian reptile, e.g. snake (*Tropidonotus*)	+	+	+	−	
Bird, e.g. fowl (*Gallus*)	+	+	+	−	
Aquatic bird, e.g. grebe (*Podiceps*)	+	+	+	−	
Insect, e.g. silkworm (*Bombyx*)	+	+	+	−	
Insect, e.g. grasshopper (*Melanoplus*)	+	+	−	−	
OVOVIVIPAROUS:					NON-CLEIDOIC
Aquatic					
Selachian fish, e.g. dogfish (*Acanthias*)	+	o	o	o	
Selachian fish, e.g. sting-ray (*Trygon*)	o	o	o	o	
Terrestrial					
No information available for ovoviviparous saurian reptiles, gastropods, or insects					
VIVIPAROUS:					
Aquatic					
Selachian fish, e.g. shark (*Carcharias*)	o	o	o	o	
Terrestrial					
Mammal, e.g. man (*Homo*)	o	o	o	o	
Aquatic mammal, e.g. whale (*Balaenoptera*)	o	o	o	o	

+ = provided by the parent organism in sufficient quantity per egg to make one embryo.
− = not provided by the parent organism in the egg and therefore required directly from the environment.
o = not provided by the parent organism in the egg but transmitted in other ways, i.e. not required directly from the environment.

water which has to be absorbed directly from the environment in order to form the relatively wet tissues of the finished embryo from the relatively dry yolk provided by the maternal organism. There is abundant evidence that all fishes and amphibia stand in like dependence upon the aquatic environment. But starting from the observation of Noble & Brady that the eggs of another species of *Amblystoma* (*opacum*) develop on land in damp leaf-mould, we find that many devices are resorted to by certain groups of amphibia to provide enough water for their embryos while yet not allowing them to develop actually *in* pond or river water. Some of these are described in the reviews of Wunder and Noble. Like the eggs of *A. opacum*, such embryos certainly get the necessary water, but often in remarkable ways. Thus the Japanese frog, *Rhacophorus schlegeli*, lays its eggs in holes in the ground, enclosed in a stable foam which holds much water for long periods. Other species, such as *Natalobatrachus bonebergi*, attach their egg-masses to rocks just above water-level in situations where the humidity of the air must always be considerable. Siedlecki, in his well-known paper on the Javanese tree-frog, *Polypedates reinwardtii*, describes how the mass of eggs is stuck between two leaves, so that when later on the central part of the jelly breaks down, an artificial pond is formed in which the larvae swim. Very many species of tree-frogs, such as *Hyla* and *Phyllomedusa*, show similar adaptations.

Buxton[1], in his book on animal life in deserts, notes it as quite remarkable that amphibia should occur at all in desert regions. It appears nevertheless that they do. Different amphibia inhabiting the Central Australian desert solve the problem of their non-cleidoic eggs in different ways. *Hyla rubella* depends primarily on a much accelerated incubation time, so that the best advantage is taken of waters in temporary pools and streams. So also the Chinese rain-frog, *Kaloula borealis*, according to J. C. Li, gastrulates in 11 hours and hatches in 3 days instead of the usual week or more. Other frogs make use of deep burrows, where humidity will remain high even when the surface has dried out (*Limnodynastes ornatus*), some lay large masses of water-holding jelly in the burrows (*Heleioporus pictus*), and some can store a very large quantity of water in their urinary bladders which may be used for moistening the eggs (*Chiroleptes platycephalus*). In the case of *Heleioporus* the eggs are believed to develop to the tadpole stage and then to "hibernate" till the advent of rain, when the membranes burst and the larvae hatch.

Water, either partly from the parent's bladder, or contained in the sand, is certainly absorbed by the eggs of many chelonian reptiles (see *CE*, p. 898, and lately Imamura). According to new observations (Cunningham & Hurwitz; Cunningham & Huene; Cunningham, Woodward & Pridgeon) the water-intake of the eggs of the turtle, *Caretta caretta*, accounts for the 50% increase in weight which they show during their development, and the eggs of the diamond-back terrapin (*Malaclemys centrata*), of an initial wet wt. of 10·58 gm., gain 3·07 gm. water while they lose 0·32 gm. in combusted organic substance. These figures are incorporated in Table 6. The content of inorganic substance remains of course unchanged.

It is not quite clear whether we are right in placing all the eggs of saurian reptiles in a definitely cleidoic position as regards water in the diagram. Some species, such as the gecko (Goeldi), are unambiguous, and most lizard eggs are independent of water. But Portmann[2] has made a special examination of the eggs of the grass-snake, *Tropidonotus natrix*, from this point of view and finds that in damp earth there is an uptake of water by the eggs (gain in weight) accompanied by successful incubation, while under the same conditions as hens' eggs there was a rapid loss of water and all the embryos died. The water may enter as vapour; in a saturated atmosphere, an even balance of weight is maintained.

Portmann thinks that many other reptiles occupy such a position, and this would agree with the work of Cunningham & Hurwitz on the American fence-lizard, *Sceloporus undulatus*, and with the old observations of Thilenius on the tuatara (*Sphenodon*; *Hatteria*).*

The water requirements of bird embryos in their cleidoic eggs will be discussed presently in relation to the normal mortality curves which they show (below, p. 64). Here it need only be recalled that (*CE*, p. 876) the water-loss from a bird's egg is proportional to the humidity of the external air. It was formerly thought that the eggs of aquatic birds, such as the grebe (*Podiceps*), which have shells impregnated with wax or oil, were impermeable to water or less permeable than hens' eggs, but this has been contested by Portmann & Jecklin. In deserts the water conservation in birds' eggs must be a problem of great difficulty, and it is interesting to read in Buxton's book that the various species of sandgrouse (*Pterocles*) which inhabit nearly every part of the Great Palaearctic desert (from the Atlantic coast of Africa to the borderland between the Gobi and China) fly regularly many miles to water and return to their nests carrying it in their soaked feathers. The young certainly depend on this for their water, and it is likely that the eggs are watered in the same way. In this connection the "pigeon milk" as a source of water for nidicolous birds should be remembered (see below, p. 75).

Insects present an interesting special case. In structure and metabolic qualities (see below) their eggs are cleidoic, but at any rate in some cases the embryos succeed in getting a certain amount of water from the environment (cf. the previous discussion of this, *CE*, p. 905). For insect embryos, as for those of birds, there is an optimum humidity. In an interesting review (which should be consulted for the special literature), Buxton^2 shows that for many insect eggs, at any rate (e.g. the grasshopper *Camnula pellucida*), the water-loss is directly proportional to the saturation-deficiency.† Percentage hatchability closely follows this water-loss. It may be significant that the eggs of many insects normally occur in places where the humidity is very high and that most of them do not suffer from exposure to saturated air, though a few are known to do so. Excessive dryness, on the other hand, is more commonly fatal; in this case, the embryo may be killed directly, or the shell so hardened that hatching is impossible. Certain eggs, such as those of the South African *Locusta pardalina* (Lounsbury) or the Australian springtail, *Sminthurus viridis* (Davidson), enter a state of dormancy if severely dried, and they may then live for several years and resume their development when wetted. Other eggs, such as those of the yellow-fever mosquito, *Aëdes argenteus*, resist drying, but in a different way, for if drying occurs when they are nearly ready to hatch, the eggs do not shrink, for the membranes have become impermeable to water. In this state they may remain hatchable for 7 months.

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* But of course such embryos have no opportunity of getting rid of nitrogenous waste-products, see on, p. 50.

† Saturation-deficiency is the amount of water vapour which would have to be added to a sample of air to saturate it, without altering the temperature.

Since the time of Réaumur in 1740 it has been known that the eggs of sawflies, which are inserted into the leaves of plants during their development, perceptibly swell. Réaumur described such eggs as doubling in size as they develop, and queried whether the shell of the egg might act as a "placenta" to draw nourishment from the leaf to which it is fastened. There are numerous modern observations to the same effect (Blunck; Johnson; Roonwal[1]), and dyes have been shown to enter the eggs from the plant. Johnson, in a very detailed study of the development of the egg of the capsid bug *Notostira erratica*, observed that as the eggs swell the yolk extrudes as a plug under the operculum or micropylar cap. He then measured the water-content and found it to rise from 0·052 mgm./egg initially to 0·148 mgm./egg at hatching; correspondingly the dry weight diminished from 0·059 to 0·050 mgm./egg. These figures are included in Table 6.

Bodine[2], however, was the first to show by direct measurement that even in the case of insect eggs lying freely in earth there may be an intake of water. The weight of an egg of the grasshopper, *Melanoplus differentialis*, rises from about 2·5 to 4·7 mgm.; this is due to the imbibition of water. The process remains stationary during diapause. We cannot incorporate these figures in Table 6, as the loss of organic substance was not known. This work was subsequently confirmed on *Locusta migratoria* (Roonwal[1]) and *Popillio japonica* (Ludwig[2]).

The exact mechanism of water-intake in insect eggs remained unclear till recently, especially as Jahn[1] showed that the egg-membranes of the grasshopper are impermeable to salts such as ferricyanide and ferric chloride. Slifer[7], however, has shown that the *Melanoplus* egg has a small, circular, specialised area in the yellow cuticle located at the posterior end; this has been named the "hydropyle" and through it the water exchanges go on. If this hydropyle is covered with any material impermeable to water, the development may be stopped or retarded, although the rest of the surface of the egg is fully permeable to gases. How far this mechanism is present in eggs of other insects and even of other arthropods is discussed in Slifer's paper, to which the reader is referred.

There still remains the further problem of what it is which creates the inward flow of water through the hydropyle. Perhaps the yolk has "deliquescent" properties. It is interesting in connection with this that McLeod states that certain plants (e.g. *Reaumuria hirtella*) secrete salt crystals which deliquesce at night and so provide water which the plant absorbs. It should also be added that though there is evidence that water may enter at the hydropyle, there is no evidence that any nitrogenous waste-products can escape there.

There can be little doubt that insect eggs take advantage of the "micro-climates" of small crevices, etc., where the humidity and temperature are different from the surrounding main bodies of air (Leeson & Mellanby; Uvarov).

On the other hand, many insect eggs lose water continuously during development. Thus Tuleschkov found that the water-loss of the eggs of the moths *Lymantria dispar* and *monacha* was of the order of 30 mgm. on 250 mgm. in 2 months.

1·63. The direct transition from the non-cleidoic state to ovoviviparity

Since the first establishment of Table 5 (as *CE*, Table 260) our knowledge of what it implies has been greatly furthered by the excellent work of Ranzi and his collaborators, who have been able to demonstrate, especially in the selachian fishes, all the stages between dependence on the environment as regards water and salts, through approximate equilibrium, to dependence on the maternal organism as regards water, salts and organic substances.

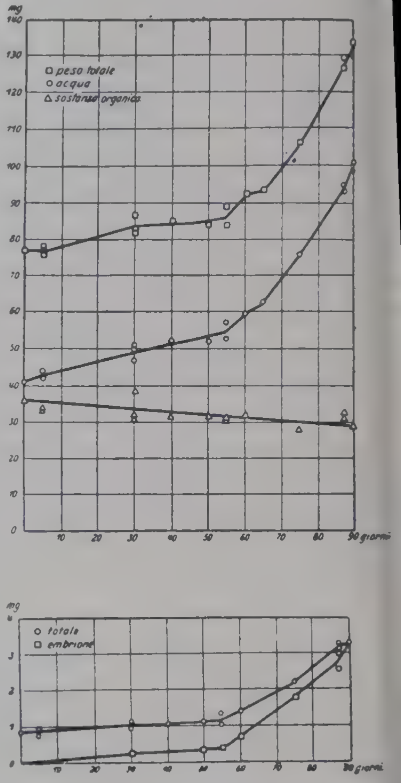

Fig. 6. Dependence of the cephalopod embryo on the environment.

Ranzi's investigations began with the eggs of cephalopods. He was able to show[6] that the weight of the whole egg of the squid *Sepia officinalis* rises during development from about 75 mgm. to about 135 mgm., and that this is accounted for largely by the intake of water. But the total ash rises also from just under 1 mgm. to about 3½ mgm., showing that the cephalopod egg is dependent also on the ash of the sea-water (see Fig. 6). It is not provided either with sufficient water or sufficient inorganic materials for the production of one complete embryo. Following the equation earlier obtained for the trout and the axolotl (shown in Table 6), a similar equation could be drawn up for the squid. This striking intake of water and inorganic material from the environment is doubtless aided by the strong ciliary currents which, as Ranzi[1] had previously shown, circulate from an early stage of cephalopod development within the perivitelline space. In view of similar findings on the

Table 6. *Dependence of eggs on the aquatic environment*

	Yolk (wet wt.) gm.	+ External water gm.	+ External ash gm.	= Completed embryo (wet wt.) gm.	+ Organic substance combusted gm.	
Trout (*Salmo*)	1·0	0·7	—	1·56	0·14	Gray[1]*
Axolotl (*Amblystoma*)	1·0	0·74	—	1·88	0·065	Dempster
Squid (*Sepia*)	1·0	0·78	0·033	1·727	0·089	Ranzi[6]
Squid (*Loligo vulgaris*)	1·0	0·25	0·008	1·155	0·105	Stolfi[1]
Squid (*Loligo bleekeri*)	1·0	3·38	0·113	4·52	Trace	Kamachi[3]
Dogfish (*Scyllium*)	1·0	1·115	0·029	2·047	0·097	Ranzi[12]
Torpedo (*Torpedo*)	1·0	1·086	0·014	1·972	0·128	Ranzi[12]
Atlantic salmon (*Salmo salar*)	1·0	1·498	—	2·34	0·16	C. R. K. Allen
Turtle (*Malaclemys*)	1·0	0·26	—	1·41	0·03	Cunningham *et al.*
Capsid bug (*Notostira*)	1·0	0·86	—	1·78	0·081	Johnson

* Trout eggs take in about 18% of their water immediately on being laid, whether fertilised or not (Manery & Irving). Teleostean embryos in general probably take in far more after hatching than before it (Stroganov; Manery, Warbritton & Irving; Irving & Manery; C. R. K. Allen; S. Smith[1]; Aoki; Bogucki[3]).

eggs of echinoderms, Needham[3] suggested that such marked dependence on the salts of the environment might be a very important barrier to penetration into fresh-water and so help to explain the absence from it of groups such as the echinoderms and cephalopods.*

Ranzi's results on *Sepia* were confirmed for the squid *Loligo* by Stolfi[1] (see Table 6) and independently on a Japanese species of the same genus by Kamachi[3]† (see Table 6). An abundant water-intake was further observed and measured by Jecklin in his study of other cephalopods, especially the octopus, *Alloteuthis*, and the squids, *Sepiola* and *Sepietta*. Kamachi[3], estimating separately a number of ions, found an intake of Ca″, Mg″, SO_4'', Cl′ and, to a lesser extent, Cu and F′. Kumon, working on the eggs of the gastropod *Hemifusus tuba*, obtained substantially the same results. This line of work was carried much further by Ranzi[17] with the help of spectrophotometric methods. He found that the squid embryo can not only concentrate the Na, K, Ca, Mg and P of the sea-water, but also rarer ions such as Cu, Fe, Mn, Mo, V, B, Li, Ni, Si, Sr, Ti and Zn, and even established[23] that a very large proportion of the Cu needed for making the amount of haemocyanin with which the embryo hatches is contained in the sea-water and not given to the egg by the maternal organism. An egg contains 0·8γ Cu at the beginning of development; it hatches with 12γ. This is not the largest increase (Ca increases eighty times), but in view of the essential nature of the blood-pigment, it is very remarkable. In a later paper Ranzi[24] showed that haemocyanin begins to be formed in considerable quantities about the 60th day of incubation (Naef stage XV–XVI), and correspondingly the absorption of copper from the sea-water occurs mainly from this time onwards.

In none of the cephalopods studied is there any substantial change in osmotic pressure during development (d'Amora; Jecklin).

The work of Ranzi and his collaborators on the elasmobranch fishes has been brought together in several reviews (Ranzi[19,20,22]). This group of animals offers special opportunities for the study of the dependence of the embryo upon the environment, for it includes purely oviparous forms (such as the dogfish *Scyllium canicula*), ovoviviparous forms (such as *Mustelus vulgaris*) showing all grades of relationship between the embryo and the maternal uterus, and the viviparous forms (such as *Mustelus laevis*) in which there is a kind of yolk-sac placenta.

* A theoretical possibility here is that an egg developing in fresh-water might still be dependent on one particular ion. This seems to be the case as regards calcium with certain molluscs such as *Limnaea* if the circumstantial evidence of S. Bloch is correct, but it is difficult to believe that the fresh-water pulmonates should have any non-cleidoic qualities in view of their probable secondary entry into aquatic life. Yet Burdach long ago, in the dissertation quoted on p. 576, found a little more ash in pond-snail eggs at hatching than when newly deposited. Indications are not wanting that an uptake of calcium may occur even in amphibia. Thus the figures of Iseki & Kumon for the giant salamander *Cryptobranchus japonicus* show a rise of from 5 to 12 mgm. CaO 100 eggs during development, and Takamatsu's for *Hynobius nebulosus* show a rise in total ash. We can hardly dismiss these entirely as due to technical error when there is direct evidence that tadpoles take up calcium salts from the water in which they are developing (Luciani, Filomeni & Severi) and that certain amphibia, such as the toad *Bufo marinus*, will not develop at all in water devoid of calcium (Takano & Iijima). Crayfish can take up Ca″ from fresh-water for calcification (Maluf[4]).

† Kamachi thought that the egg-membranes supplied some of the water, salts and even nitrogen to the embryo, but the figures of Stolfi to the contrary carry more conviction.

Ranzi[12] first showed that the development of oviparous forms such as *Scyllium canicula* exactly resembles that of other marine eggs such as the squid or octopus egg (Table 6) in obtaining some 60% of the inorganic substance which it requires, and some 70% of the water, from the aquatic environment and not from the maternal organism. Now in all the ovoviviparous and viviparous forms this process is repeated[13], as is shown by the figures in Tables 7 and 8, but in addition to this, the negative balance of organic substance caused by combustions is gradually changed over into a positive balance as the embryo comes from species to species more closely in contact with the maternal organism. Thus in the spiny dogfish, *Acanthias vulgaris*, there is still a loss of organic substance during development; in *Acanthias blainvillei* the loss by combustions and the gain from the maternal organism just balance, so that there is neither a total loss nor a total gain; and in the smooth dogfish, *Galeus canis*, *Mustelus antarcticus*, *Myliobatis bovina*, etc., we see a gradually increasing deposition of organic substance from the maternal organism in the egg (yolk + embryo) during development. This reaches its maximum in *Pteroplatea micrura*, where the gain in organic substance is some five thousand per cent. At the same time it may be noticed that those elasmobranchs which depend very largely on the maternal organism for organic substance rather than on the yolk, tend also to depend on it for inorganic substance and water, rather than on the sea-water, to a much larger extent than those which carry on their development outside the maternal organism. For example, the water-intake of *Scyllium* is about 200% of the initial store, but in the case of *Myliobatis* and the sting-ray *Trygon* it is several thousands per cent.

Table 7. *Dependence of eggs on the uterine environment in ovoviviparity and viviparity*

	Yolk (wet wt.) gm.	+	External water gm.	+	External ash gm.	+	External organic substance gm.	=	Completed embryo (wet wt). gm.
Ovoviviparous:									
Dogfishes									
(*Galeus canis*)	1·0		1·05		0·078		0·05		2·18
(*Mustelus antarcticus*)	1·0		6·3		0·013		0·57		8·0
(*Mustelus vulgaris*)	1·0		12·18		0·36		1·77		15·31
(*Trygon violacea*)	1·0		52·1		1·1		8·0		62·2
(*Myliobatis bovina*)	1·0		52·0		1·1		8·2		62·3
Viviparous:									
(*Mustelus laevis*)	1·0		26·9		0·95		5·4		33·25

Successful correlations were also made by Ranzi[13] between the degree of dependence shown by the egg and (*a*) the nature of the uterine fluids, (*b*) the structure of the uterine wall, and (*c*) the extent to which the stored substances in the liver of the maternal organism are depleted during development. These phenomena may be taken in turn.

As Table 8 shows, there are considerable variations in the composition of the uterine fluid bathing the egg-cases while the embryo develops. In *Torpedo* it is abundant but dilute, in *Scymnus* and *Acanthias* it is less abundant but more concentrated, and at the change-over point between loss or gain of organic substance in the egg, it has a concentration of organic substance of about 3%.

Table 8. *Balance sheet of developing elasmobranch embryos in oviparous, ovoviviparous and viviparous conditions*

Species	*t*	*e*	*E*	Change %	*w*	*W*	Change %	*a*	*A*	Change %	*o*	*O*	Change %	*ouf* %	*uw*	*R*
Oviparous:																
Scyllium canicula	6	1·3	2·7	+108	0·68	2·15	+216	0·013	0·051	+292	0·61	0·48	−21	—	—	—
Ovoviviparous:																
Torpedo ocellata	4	6·8	13·4	+103	2·94	10·31	+300	0·06	0·15	+150	3·78	2·91	−23	1·2	Ib	8·5
Torpedo marmorata	6	14·4	27·3	+91	6·56	21·85	+233	0·14	0·40	+186	7·68	5·06	−31	1·5	Ib	9·1
Scymnus lichia	10	130	188	+45	65	131	+100	2	8	+30	63	49	−22	1·6	Ia	6·3
Centrophorus granulosus	10	308	360	+17	142	274	+94	3·4	12	+253	162	74	−54	1·8	Ia	—
Acanthias vulgaris	10	23	41	+78	14·2	35	+147	—	—	—	8·6	5·2	−40	2·4	Ia	—
Acanthias blainvillei	9	19·3	29·6	+53	8·4	17·9	+88	0·2	1·8	+1250	10·7	10·8	+1	2·8	Ia	13·6
Galeus canis	10	46·9	102·2	+118	23·5	72·6	+210	0·45	3·6	+700	23	25·5	+11	4·9	II	—
Mustelus antarcticus	10	4·4	32·3	+635	2·1	29·8	+1480	0·05	0·6	+1100	2·3	4·8	+110	7·1	II	—
Mustelus vulgaris	10	3·9	60·6	+1450	1·9	49·8	+2520	0·55	1·5	+173	1·9	8·9	+369	5·1	II	56·1
Pteroplatea micrura	?	—	—	—	—	—	—	—	—	—	0·2	10	+4900	—	III	—
Myliobatis bovina	4	7·2	450	+6150	5·2	381	+7200	0·08	8·0	+990	1·9	61	+3120	—	III	—
Trygon violacea	2	2	118	+5800	0·9	97	+10000	0·2	2·1	+950	0·9	16	+1680	13·3	III	51·3
Viviparous:																
Carcharias glaucus	10	—	—	—	—	—	—	—	—	—	3·4	32	+840	—	II	—
Mustelus laevis	10	5·5	189	+3340	2·6	152	+5750	0·7	5·3	+660	2·8	32	+1050	9·1	II	37·4*

* In *M. laevis* the weight of the maternal liver may rise again later in development, R is here calculated up to the time when it is at its lowest, i.e. when the embryo weighs 13 gm. This is a further argument for the greater efficiency of viviparity.

Key to Table 8

t time of incubation or gestation in months.
e wt. of egg at the beginning of development, gm.
E wt. of embryo at the end of development, gm.
w wt. of water in the egg at the beginning of development, gm.
W wt. of water in the embryo at the end of development, gm.
a wt. of ash in the egg at the beginning of development, gm.
A wt. of ash in the embryo at the end of development, gm.
o wt. of organic substance in the egg at the beginning of development, gm.
O wt. of organic substance in the embryo at the end of development, gm.
ouf organic substance in the uterine fluid, %.
uw type of uterine wall.
R $100\left(1-\frac{FM_0}{MF_0}\right)$, where F_0 is the weight of the maternal liver at the beginning of development and F the weight at the end, M_0 the weight of the maternal body at the beginning of development and M the weight at the end.

Percentages are of the initial value.

When the dependence on the maternal organism is more marked, the uterine fluid becomes turbid and contains formed elements in suspension, as in *Trygon*; it then may reach 13·3 % organic substance. Ranzi[14] found that in some cases, at any rate, leucocytes transport fat from the tissues to the uterine fluid, where later they may disintegrate, leaving the fat in suspension. In *Trygon* uterine fluid contains 8 % fat. The embryo absorbs the uterine fluid through the yolk-sac and also probably in some cases through its enlarged gills; later on injections of Chinese ink show that it passes into the digestive tract through the mouth and spiracles. Proteases have been found in the uterine fluid of *Torpedo* and *Mustelus* (Saviano), but they do not seem to act at the *p*H of the fluid itself, and perhaps only do so when the fluid has reached some part of the intestinal tract. At an early stage (length 46 mm.) the digestive glands of the *Torpedo* embryo are all functional (Pitotti[1]), and while the uterine fluid is being digested in the stomach, the yolk is being digested in the intestine. Histological details of the selachian yolk-sac will be found in a paper by Macé.

It seems that nourishment of the embryo by means of the elasmobranch "histotrophe"[*] is so efficient as to exceed the efficiency of the viviparous types, in which the yolk-sac actually invades the wall of the uterus. As Table 8 shows, the uptake of water, ash and organic substance in *Mustelus laevis*,[†] the type specimen of selachian viviparity, in percentage of the initial stores, is actually rather less than in some of the most successful ovoviviparous types, such as *Myliobatis* and *Trygon*. There may, however, be many other reasons which have led to the evolution of true viviparity in these fishes, and indeed Ranzi pointed out that the speed of development and absorption is much greater in *M. laevis* than in *M. vulgaris* or *antarcticus*.

This brings us to the question of the nature of the uterine wall. Ranzi's researches show[19] that this differs among the different groups. In *Torpedo* the wall has a great number of villi of one-layered epithelium which contain glands but no mucus-secreting cells (see Fig. 7). In *Scymnus*, *Centrophorus*, *Acanthias*, etc. there are many villi as before (see Fig. 8), but the epithelium is many-layered, and there are a few mucus-secreting cells. In *Mustelus vulgaris* and *Galeus canis* the uterine wall is much less villous, but its smoother surface contains very many cells which secrete a mucous material into the uterine fluid, thus raising its protein-content though still it contains little fat. The protein seems to be mainly mucoprotein, but in addition to this bound carbohydrate, there is about 0·2 % free reducing sugar. As in all other elasmobranch tissue fluids there is abundant urea present. Finally, in *Trygon*, where the uterine fluid is particularly rich in nutrient substances, the villous condition of the uterine wall is carried to an extreme (see Fig. 9). The epithelium is here many-celled, and there are many glands secreting protein and fat. The liquid contains 0·5 % free reducing sugar.

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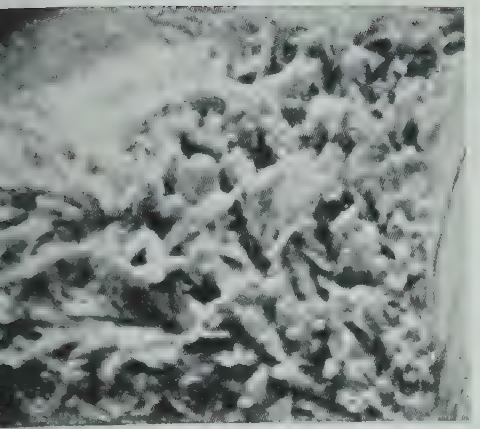
[*] See on, p. 81, in connection with mammals, and cf. *CE*, p. 1492. Hartman[2,3] sees in these uterine secretions the evolutionary origin of menstruation.

[†] One can hardly avoid mentioning here the story, told in full by Haberling, that this fish is the γαλεὸς λεῖος of Aristotle, *Hist. Anim.* 565 a & b, who knew it to be viviparous, i.e. placental; but was not believed until the rediscovery of this fact in 1842 by Joh. Müller.



7. The uterine wall of *Torpedo*.

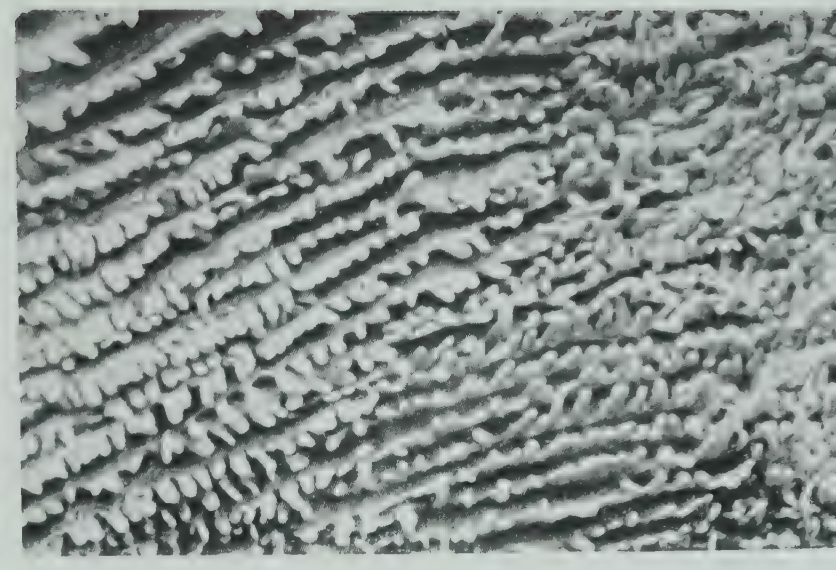


Fig. 8. The uterine wall of *Centrophorus*.

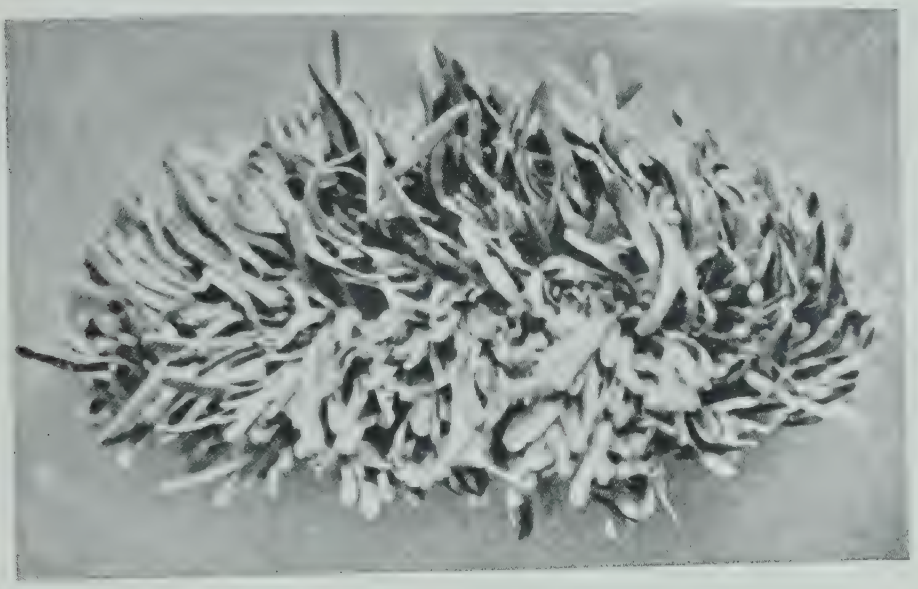


Fig. 9. Villi of the uterine wall of *Trygon*.

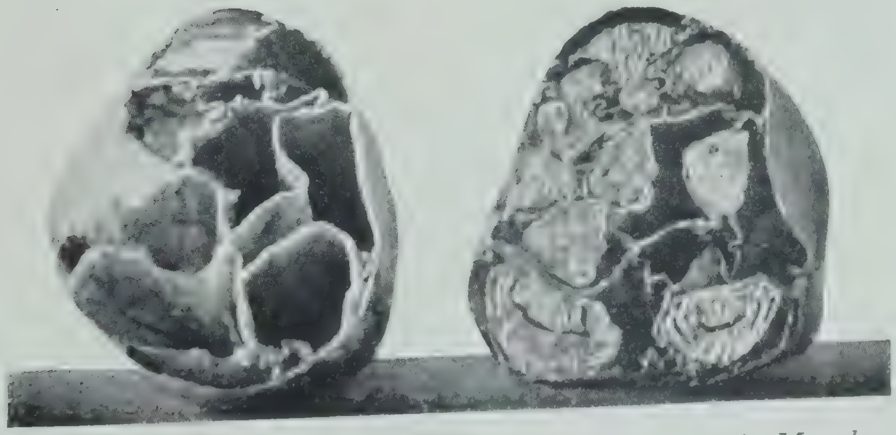


Fig. 10. Egg-cases forming compartments in the uterus in *Mustelus*.

In the true viviparous cases, such as *Mustelus laevis*, the embryos are separated by compartments (Fig. 10) and there is a specially modified condition of the yolk-sac so that it burrows into the maternal tissues (see Figs. 11, opp. p. 46, and 12).* Correspondingly the content of organic substance in the uterine fluid is diminished with respect to the ovoviviparous forms (Table 8) and fat in particular is missing from it (only 1·25 %). Calzoni[2] has described the placenta of *Carcharias glaucus*.

All these relationships will be found summarised in Fig. 13. The rectangles left of the ordinate indicate the extent to which the egg receives organic substances from the maternal organism; a negative sign means that this does not occur. The rectangles right of the ordinate indicate the organic substance content of the uterine fluid (dotted lines show probabilities). The black rectangles right of the ordinate indicate the degree to which the maternal liver is reduced during development (see below), and side by side with each group is placed a diagram of the histological structure of the uterine wall, as described above.

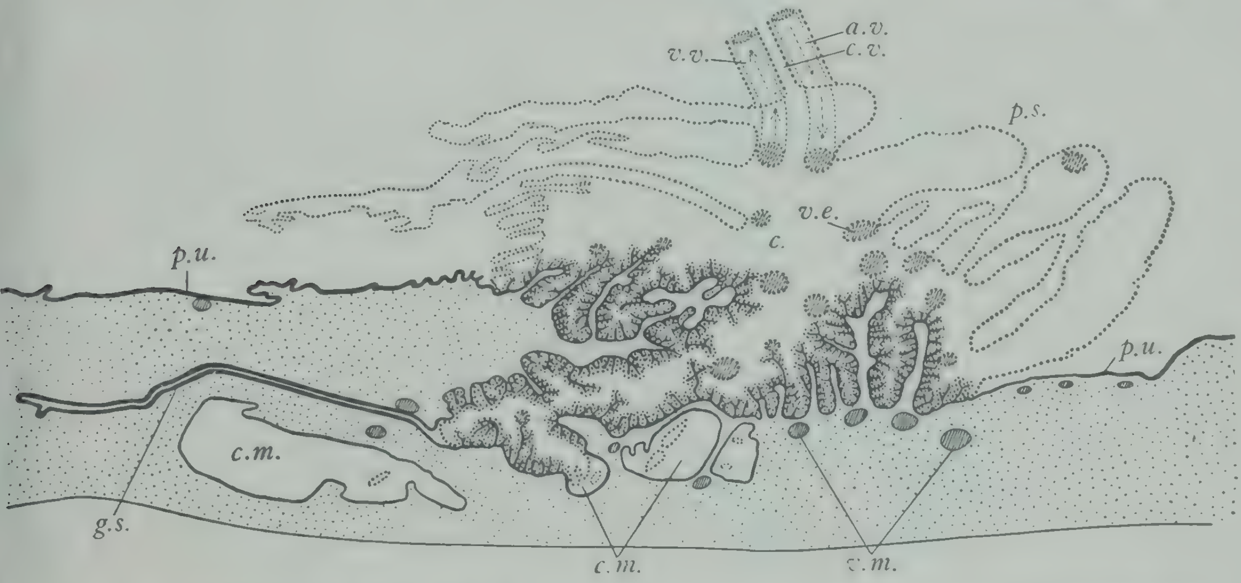


Fig. 12. Epithelio-chorial placenta of *Mustelus* in section. *a.v.*, vitelline artery; *c.*, yolksac cavity; *c.m.*, lumen of mucus-secreting glands; *c.v.*, duct from yolk-sac to intestine; *g.s.*, gland lumen; *p.s.*, yolksac wall; *p.u.*, uterus wall; *v.e.*, foetal blood vessels; *v.m.*, maternal blood vessels; *v.v.*, vitelline vein.

The last of these questions is the reduction of the maternal liver. Ranzi[15] noticed that there was a parallelism between the degree of dependence of the eggs on the mother and the degree to which the liver is reduced in size during their development. The last column in Table 8 shows values for this by a factor, *R*. The rise and fall in liver weight appears to have a close connection with such endocrine glands as the pituitary (Ranzi[21]; Comes; Castigli), the thyroid (Zezza; Guariglia) and the suprarenals (Fancello; Pitotti[2]; Ranzi[22]). It is undoubtedly integrated into the selachian endocrine cycle of reproduction, a discussion of which with relation to the similar cycle in mammals has been given by Ranzi[19]. In mammals the liver changes are much smaller (Biereigel), suggesting that the effects of pregnancy on the adult organism are there less.

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* It is noteworthy that the placenta of *Mustelus laevis* resembles the most impermeable placenta type among the mammals; in the Grosser classification, the epithelio-chorial (see p. 81).

The exact nature of the liver changes has also been studied. According to Ranzi & Zezza (confirmed by Falkenheim) there is no destruction of cells during the rise and fall of liver size, simply a swelling followed by a shrinking, so that, as embryonic development progresses, the number of nuclei visible in a single standard section of the maternal liver constantly rises. The number of visible lipoid droplets correspondingly falls.

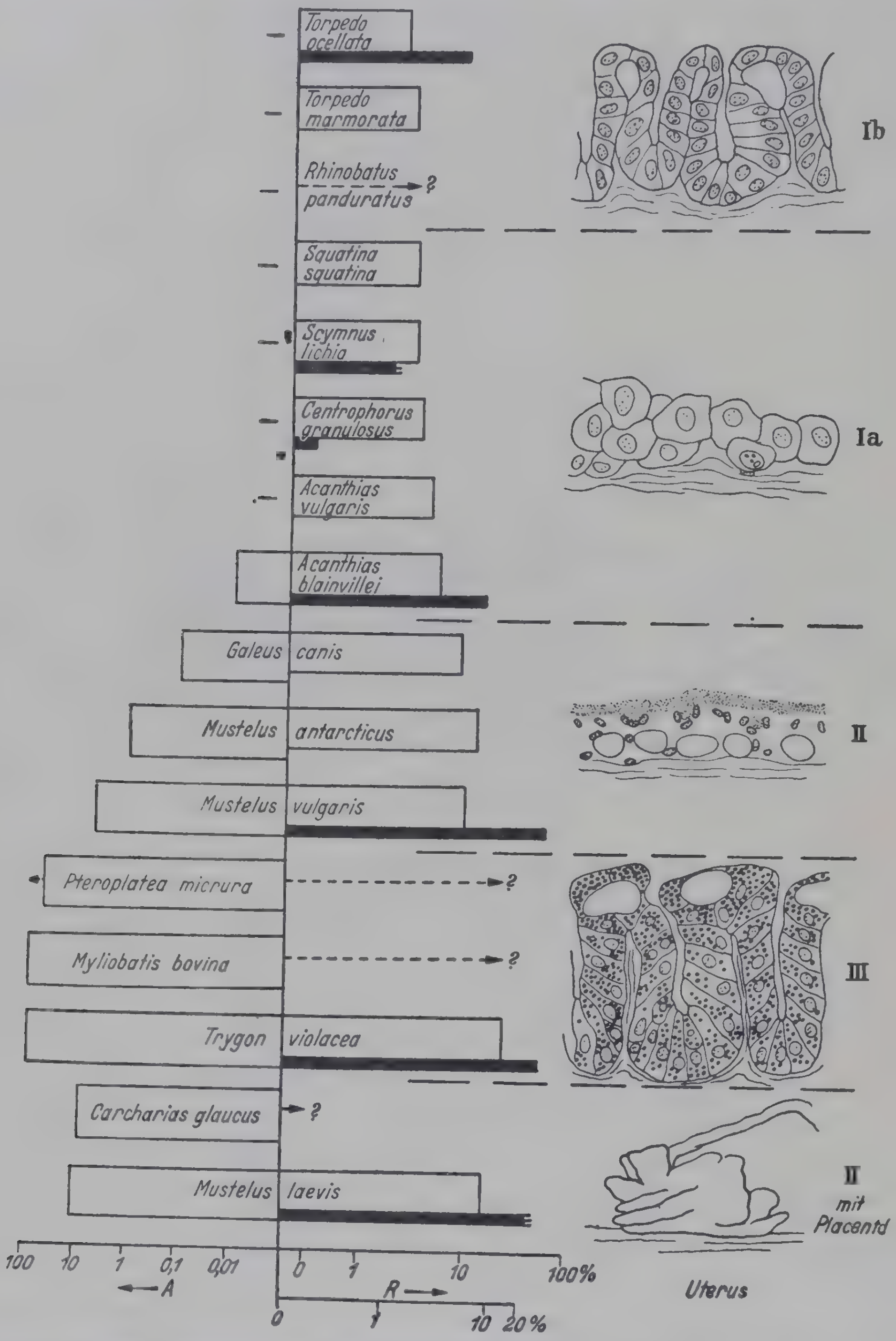
Torpedo ocellata
Torpedo marmorata
Rhinobatus panduratus ?
Squatina squatina
Scymnus lichia
Centrophorus granulosus
Acanthias vulgaris
Acanthias blainvillei
Galeus canis
Mustelus antarcticus
Mustelus vulgaris
Pteroplatea micrura ?
Myliobatis bovina ?
Trygon violacea
Carcharias glaucus ?
Mustelus laevis
Ib
Ia
II
III
II mit Placenta
100 10 1 0,1 0,01
← A
0 1 10 100%
R →
0 1 10 20%
Uterus

Fig. 13. Diagram summarising the relations between maternal and foetal organism in the elasmobranch fishes.

We are not without knowledge concerning the nature of these lipoid substances. Leaving out the earlier work on the subject, previously reviewed (*CE*, p. 1180), may be said that André established the fact that livers of elasmobranch fishes gestation contain more oil than when non-gestating, e.g.

	% oil	
	Non-gestating	Gestating
Scyllium stellare	20	50
Galeus canis	28	47
Raia batis	22	52

ndré & Canal then examined the proportion of unsaponifiable matter in the ver oils from adult and young fishes in the pilgrim shark (*Cetorhinus maximus*).

	Fat %	Unsaponifiable %	Cholesterol %	Squalene and pristane %
Liver of adult fish	50	50	2	48
Liver of young fish	64	36	22	18

he young fish has therefore less unsaponifiable matter but relatively more nolesterol. They then compared the liver oil with the egg oil in *Centrophorus ranulosus*.

	Fat %	Unsaponifiable %	Cholesterol %	Squalene and pristane %
Liver of adult fish	9	91	1	90
Undeveloped egg	50	50	5	45
Liver of embryo	33	66	4	62

hus again the embryonic liver has less unsaponifiable matter and relatively more holesterol. In both animals the embryo and young fish is richer in neutral fat, nd this is especially marked in the eggs of *Centrophorus*, where there is as much aponifiable as unsaponifiable matter. André & Canal believed in a conversion f clupanodonic acid to cholesterol and squalene, but of this there is no metabolic oof. Stolfi[2] found that the iodine values of the fat of the maternal liver in *rygon* are much higher than those of the uterine fluid (especially rich in fat, we have seen).

	Iodine value
Maternal liver fat	131
Uterine fluid fat	73
Embryo tissue fat	69

may therefore be concluded that the fatty acids undergo saturation in passing the embryo, but no information indicating how or where this takes place is et available.

If the general line of argument so far followed is correct, it might be expected at eggs of related species of different habitat would contain less ash if from ea-water, more if from fresh-water. The only comparative estimations of this ind are contained in a note by Buşniţă & Gavrilescu, who analysed teleost eggs carp and herring) from different habitats.

	Ash % dry wt.	
	Lake Greaca (fresh)	Lake Tasaul (brackish)
Cyprinus carpio	4·08	3·89
	River Danube (fresh)	Black Sea
Alosa pontica	6·50	5·74

hese differences they regarded as significant.

The foregoing investigations are the most complete which have so far be made on the relations between maternal organism and embryo in any no mammalian group. Viviparity is certainly a very different matter in an aquat environment to what it is in a terrestrial one, as we shall shortly see (p. 51 and in marine conditions it originates probably as a purely protecting mechanis However, the same story is found in most groups of animals among the lowe vertebrates, and it is worth while to pause for a moment to see how it works o in other fishes and amphibia.

From the excellent review of Wunder[1], which summarises much rathe inaccessible literature in the field of natural history, we find that among th teleosts too there has been a move towards ovoviviparity in certain species While there seems to be nothing corresponding to the yolk-sac placenta o *Mustelus laevis* among teleosts, the ovoviviparous eel, *Zoarces viviparus*, secrete a very fatty and mucous uterine fluid, which places it in a position analogous t *Trygon*. Stuhlman's analysis (1887), the only one ever made, described this fluid as "rich in nutritive substances". In the Cyprinodonts, such as *Girardinus* and *Poecilia*, the eggs develop while still in the ovary, and the embryo hatches from the egg-case later on in the oviduct. Remarkable adaptations to this ovarian life have been described by C. L. Turner; extensive ribbon-shaped proctodaeal processes occur in the embryos of both fresh-water (e.g. *Goodea bilineata*) and marine (e.g. *Parabrotula dentiens*) forms. Some Cyprinodonts seem to be just on the borderline between oviparity and ovoviviparity, for Amemiya & Murayama noted that the eggs of *Oryzias latipes*, which normally develop outside the parent's body, can sometimes do so within it. Unfortunately few biochemical investigations have yet been made from which we can draw any conclusions about the role of maternal nutrition in such cases. C. D. Hsiao, however, working on the deep-sea teleost *Sebastes marinus*, found that, although the incubation is ovarian, there is a pronounced fall in the dry weight of the developing egg, indicating little or no maternal contribution. The other extreme is represented by a cyprinodont teleost *Heterandria formosa*, studied by Scrimshaw, also with ovarian incubation. In this remarkable form, conditions seem to be almost mammalian, for the egg is only 0·39 mm. in diameter at fertilisation and the dry weight increases from 0·017 mgm. to nearly 10 mgm. at birth. A typical teleostean oil-drop is present, occupying 90 % of the space in the minute egg, but it is not utilised until late in development. Absorption of nutritional materials is assisted by a richly vascularised expanded pericardial sac which completely envelops the embryo's head until the later stages. Evidently all grades of maternal dependence occur in teleosts just as they do in selachians. It is interesting and perhaps significant in view of what happens in selachians that in *Gambusia holbrooki* Remotti[2] observed a diminution of liver weight during gestation.

A somewhat less intimate connection may be supposed to exist in those fishes where the eggs are attached in various ways to the external surface of the body of one or other of the parents, as occurs in the fresh-water teleosts *Kurtus gulliveri* (New Guinea) and *Aspredo laevis* (South America). The most striking example of this kind of protection occurs in the Syngnathidae, of which the

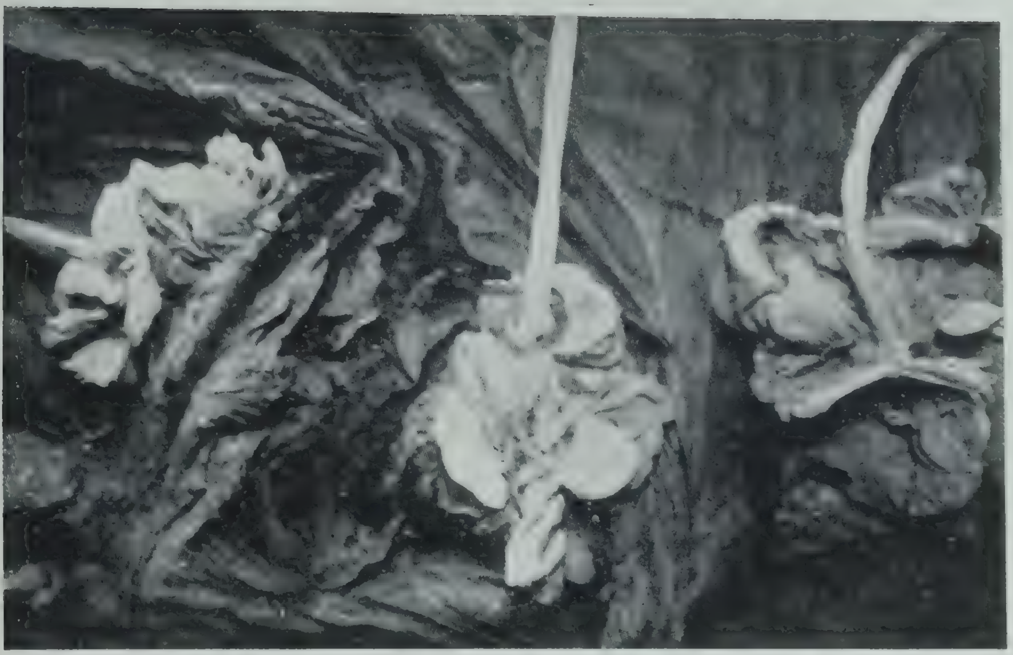

Fig. 11. Yolk-sac placentas in *Mustelus*.

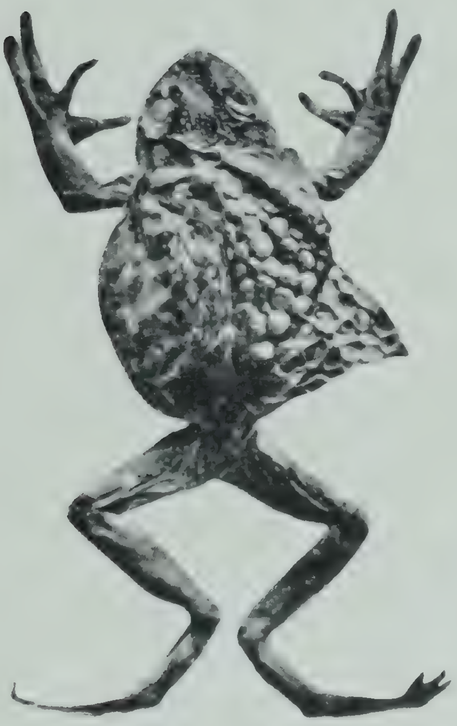

Fig. 14. Female of *Nototrema marsupiatum* with the skin of the back dissected to show the eggs.

ı-horse, *Hippocampus antiquorum*, is a classical case. Here the male fish carries eggs in a marsupial-like pouch formed of two large folds of skin either on belly or the tail. On the inside of the pouch in the pipe-fish *Syngnathus typhle* ıhn found secretory glands, as did Kolster in *Hippocampus*, and the fluid ntents of the pouch seemed to contain protein and fat, but Comes and Leiner[1], direct measurements, showed that the embryo of *Hippocampus brevirostris* ıring its development takes up water and inorganic substances, but not organic bstances, from the male organism carrying it.* It compares therefore with *ymnus* or *Centrophorus* in the selachian series. Some histochemical observations an embryo of this kind (*Syngnathus acus*), which may be of interest, are con-ined in a paper by Konopacki & Erecinski.

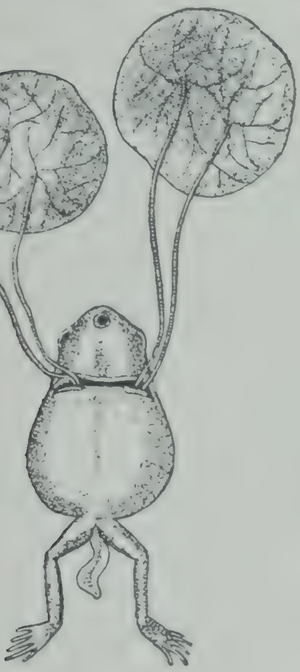

Fig. 15.

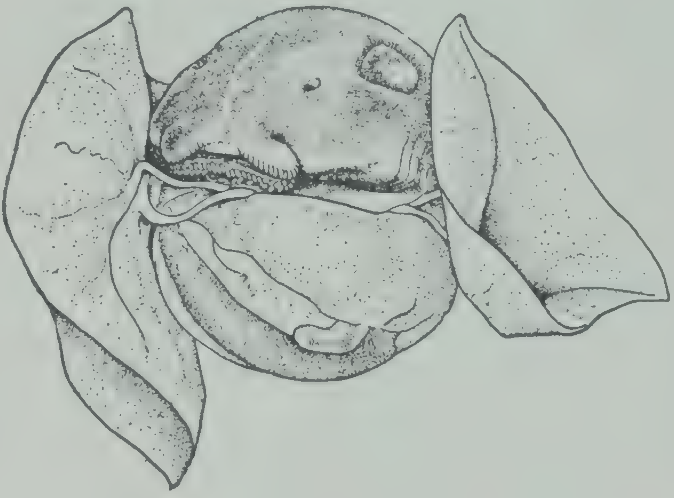

Fig. 16.

Fig. 15. Larva of *Nototrema oviferum* with leaf-like gills acting as placentas.
Fig. 16. Larva of *Nototrema marsupiatum* with bell-like gills acting as placentas.

Protection of teleostean embryos may also be carried out by the eggs being held thin the mouth of either parent, as in *Arius falcarius* and many other Silurids, as ll as among the Cyprinodonts, Trachinids, Serranids and Cichlids, etc. In *Arius* ding and digestion are in abeyance during the development of the eggs, the fish ing on its reserves, but nothing is known about the nutrition of the embryos.

When we turn to the amphibia we find a quite parallel state of affairs. From under's second review[2], which dealt with the amphibia, and from the interesting ork of Noble, it can be seen that ovoviviparity, though no true viviparity, occurs. he African frog, *Nectophrynoides tornieri*, retains its embryos to develop in the iduct, and this is common among salamanders (e.g. *Salamandra atra*). The nbryos of the South American blindworm, *Typhlonectes compressicauda*, are

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Leiner's elaborate work[2] may be consulted for an account of the composition and properties the brood-pouch liquid of *Hippocampus* (see p. 586). It is more acid than sea-water, richer in $_2$, of higher osmotic pressure, and contains a protease which Leiner pessimistically assumes provided to resorb the dead.

characterised by a special modification of the external gills, which expand in leaf-like plates. It is difficult to believe that these do not have an absorpti function, but no investigations have yet been made into the degree of dependen of any of these forms upon the maternal organism during development. Th expanded leaf-like gills may only function in gas-exchange.

Modified gills also occur in embryos of those species which protect thei developing eggs by carrying them in pouches of various kinds, similar to th Syngnathid fishes already mentioned. This frequently occurs in tree-frogs such as *Hyla goeldii* or *evansi*, but the best example is perhaps the genus *Nototrema*, where the embryos are carried in a series of pockets on the back of the adult frog. Fig. 14 (opp. p. 46) shows a female of *Nototrema marsupiatum* with the skin of the back dissected to show the eggs, and Fig. 15 a larva of *Nototrema oviferum*, showing its vastly expanded leaf-like gills. Fig. 16 shows a larva of *N. marsupiatum*, showing the bell-like form taken by the gills. Studies of the kind made by Ranzi would be extremely welcome on an amphibian series. The tail may function instead of the gills as an organ of absorption in ovoviviparous or "marsupial" amphibia; this happens in toads of the type of *Protopipa aspera* or *Pipa americana*. Fig. 17 shows a larva of *Pipa dorsigera*, showing the enlarged and well-vascularised tail.

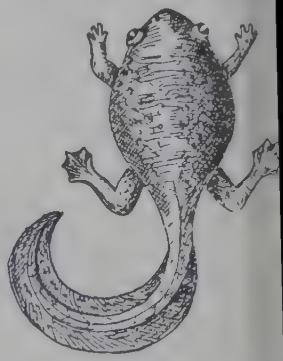

Fig. 17. Larva of *Pip* *dorsigera* with tai expanded to act as placenta.

Finally, buccal incubation occurs also among the amphibia (in *Hylambates brevirostris*), though here it more usually takes the form of allowing the eggs to develop in the vocal sac, as in the Chilian *Rhinoderma darwini*, where the tail is believed to act as an absorptive organ, though metamorphosis is fully accomplished before the larvae leave the sac.

1·64. Metabolic properties of the cleidoic state

We must now return to Table 5. There it can be seen that by far the most self-contained or *cleidoic* eggs are those which develop outside the maternal body in a terrestrial environment. Now far-reaching metabolic changes were associated with the evolution of the cleidoic egg. Closure of the egg system is naturally accompanied by difficulties in the disposal of incombustible nitrogenous waste-products. Thus it has been shown that the eggs of birds, reptiles and insects combust markedly less protein during their embryonic life in percentage of the protein initially present than do the eggs of aquatic animals such as fishes or amphibia. The figures are of the order of 3 % in the former as against 26 % in the latter (see Table 9). Similarly, of the material burned as source of energy during development 6 % is protein in the case of the chick, but 71 % in the case of the frog (see Table 10). On the other hand, the catabolism of fat is correspondingly increased in cleidoic eggs, so that, whereas the aquatic eggs of amphibia and fishes make it some 30 % of their total combustions, the cleidoic ones of birds and insects make it about 80 % (see Table 10). The same relation holds good when the fat combusted is compared with the fat initially present (Table 11).

Table 9 (corresponding to *CE*, Table 162). *Protein combusted as source of energy in % of the initial protein store*

	%	
T. Chick (*Gallus domesticus*)	4·5	
T. Silkworm (*Bombyx mori*)	3·9	
T. Sheep blowfly (*Lucilia sericata*)	0·51	Rainey; Brown
Intermediate: Turtle (*Thalassochelys corticata*)	16·5	
A. Trout (*Savelinus fontinalis*)	19·5	
A. Trout (*Salmo irideus*)	(49·3)	S. Smith[1]
A. Carp (*Cyprinus carpio*)	41·0	Nowak
A. Atlantic salmon (*Salmo salar*)	36·3	Hayes[1]
A. Plaice (*Pleuronectes platessa*)	18·3	
A. Frog (*Rana temporaria*)	29·6	
A. Salamander (*Cryptobranchus japonicus*)	27·8	Iseki, Kumon, Takahashi & Yamasaki
A. Squid (*Loligo vulgaris*)	15·0	Stolfi[1]
A. Squid (*Sepia officinalis*)	13·7	M. Cori

Averages: **T. 2·9; A. 25·9.**

T. = Terrestrial; A. = Aquatic.

N.B. (1) Detailed references are omitted for those cases already mentioned in *CE*.

(2) The figure of S. Smith may be unduly high owing to the inclusion of a starvation period after the consumption of all the yolk. A value of 32·5 %, calculated from his data, is more correct. If the period before hatching were alone considered, all catabolic losses would be very small, as the teleostean alevin hatches with most of its yolk still unused (Manery, Warbritton & Irving; Irving & Manery).

Table 10 (corresponding to *CE*, Table 161). *Material combusted as source of energy in % of the total material so combusted*

	Carbohydrate	Protein	Fat	
T. Chick (*Gallus domesticus*)	3·02	5·57	91·4	
A. Frog (*Rana temporaria*)	6·84	70·70	22·4	
T. Grasshopper (*Melanoplus differentialis*)	—	—	72·5	Boell[1]; Slifer[1,4]
T. Silkworm (*Bombyx mori*)	26	10	64	
T. Sheep blowfly (*Lucilia sericata*)	Trace	<5	>95	Rainey; Brown
Intermediate: Turtle (*Thalassochelys corticata*)	—	19	81	
A. Sea-urchin (*Paracentrotus lividus*)	21	50	29	
A. Trout (*Savelinus fontinalis*)	—	63	37	
A. Trout (*Salmo irideus*)	Trace	83·8	16·0	S. Smith[1]
A. Carp (*Cyprinus carpio*)	—	61·5	23·8	Nowak
A. Plaice (*Pleuronectes platessa*)	—	90	—	
A. Squid (*Loligo vulgaris*)	1·0	37·5	61·5	Stolfi[1]
A. Squid (*Sepia officinalis*)	—	51·5	—	M. Cori
A. Salamander (*Cryptobranchus japonicus*)	12·3	51·5	36·4	Iseki, Kumon, Takahashi & Yamasaki; Tomita & Fujiwara
Averages: **T.**	—	**6·8**	**80·7**	
A.	—	**62·1**	**32·3**	

T. = Terrestrial; A. = Aquatic.

N.B. (1) The first two sets of figures are those most accurately known.

(2) Detailed references are omitted for those cases already mentioned in *CE*.

(3) Figures for terrestrial molluscs using large amount of stored polysaccharide would be of special interest.

The cleidoic condition may also be connected with other peculiarities, perhaps the most striking of which is the rapidity of avian incubation time as compared with mammalian gestation time, i.e. the fact that it takes considerably longer to make a mammal of given birth weight than a bird of equivalent hatching weight (see *CE*, pp. 480 ff.). For a weight of 1 gm. the mammalian time is 20 % longer; for a weight of 10 or 110 gm. it is twice as long, and for a weight of 1 kg. it is three times as long. It is suggested that this is an adaptive acceleration of the developmental time, permitting rapid passage through the cleidoic state.

Table 11 (corresponding to *CE*, Table 174). *Fat combusted as source of energy in % of the initial fat store*

	%	
T. Chick (*Gallus domesticus*)	60	
T. Silkworm (*Bombyx mori*)	48	
T. Grasshopper (*Melanoplus differentialis*)	54	
T. Lackey-moth (*Malacosoma americana*)	85	
T. Sheep blowfly (*Lucilia sericata*)	44·5	Rainey; Brown[3]
T. Potato beetle (*Leptinotarsa decemlineata*)	36·5	
Intermediate: Turtle (*Thalassochelys corticata*)	34	
A. Frog (*Rana temporaria*)	28	
A. Salamander (*Cryptobranchus japonicus*)	30·8	Tomita & Fujiwara; Kataoka & Takahashi
A. Primitive salamander (*Hynobius nebulosus*)	25·5	Takamatsu[2]
A. Torpedo (*Torpedo ocellata*)	24·5	
A. Plaice (*Pleuronectes platessa*)	10	
A. Sea-urchin (*Strongylocentrotus lividus*)	20	
A. Squid (*Loligo vulgaris*)	47·6	Stolfi[1]
A. Trout (*Salmo irideus*)	51	S. Smith[1]

Averages: **T. 54·6; A. 29·6.**

T. = Terrestrial; A. = Aquatic.

N.B. Detailed references are omitted for those cases already mentioned in *CE*.

Another metabolic characteristic of birds possibly related to the cleidoic nature of their eggs is their great resistance to ketosis; we shall return later to this point (p. 62). But outstanding in birds, insects and saurian reptiles is their possession of uricotelic metabolism.

There are in the animal kingdom three main ways of excreting waste nitrogen from protein catabolism—ammonia, urea and uric acid.* In addition to these substances, many invertebrates excrete amino-acids unchanged, as if the metabolic machinery of the body which separates the amino-acids of the food into quotas for storage and quotas for combustion were inefficient (Delaunay). In certain special groups also there may be special methods or adaptations, such as the excretion of guanine by arachnids (Vajropala; Peschen)† and trimethylamine-oxide by certain teleosts (Grollmann[1]; Grafflin, Gould & Spence), but these must be regarded as minor specialisations.

That the elaborate process of the formation of the purine ring from the catabolic nitrogen had something to do with terrestrial life was early surmised,

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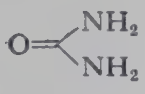
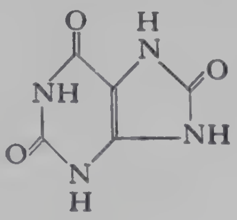
* The comparative biochemistry of nitrogen excretion has recently been the subject of elaborate tabulation and useful discussion by Heidermanns[1]. I am unfortunately unable, however, to agree with all his conclusions.

NH_3

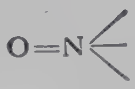
Ammonia



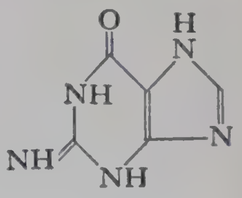
Urea



Uric acid



Trimethylamine oxide



Guanine

† The deposition of guanine in the scales of some fishes (Bethe) possibly as an excretion from nuclein catabolism, is well known, but it is not so well known that the guanine crystals from this natural source have for centuries been the basis of the artificial pearl industry, as is described by H. F. Taylor.

but the mammalia, which excrete the whole of their waste nitrogen in the form of urea, were an insuperable obstacle to so simple a generalisation. It was then suggested (*CE*, Section 9·5) that the form of nitrogen excreted by an animal depended primarily on the conditions under which its embryo had to live. Most of the data which have come to light since that time have supported this view. They may be summarised as follows. Between uric acid excretion[*] and terrestrial oviparity there is a strict correlation, ammonia and urea being associated with aquatic pre-natal life (including development within the mammalian uterus) and uric acid with terrestrial pre-natal life. An inspection of the percentage nitrogen partition in the excreta of representatives of every phylum (*CE*, Table 163) shows the strictness of the correlation.[†] Birds, saurian reptiles, insects and terrestrial gastropods alike possess the uricotelic habit; mammals, chelonian reptiles, amphibia and fishes excrete their waste nitrogen mainly as ammonia or urea; aquatic invertebrates down to protozoa excrete almost entirely ammonia.[‡]

The essential point here is that uric acid is the only excretory nitrogenous compound which can be got rid of in the solid form, requiring the loss of little water during its removal. It forms supersaturated colloidal solutions (Young & Musgrave; Young, Musgrave & Graham), from which it readily precipitates in an almost insoluble condition. In insects (Wigglesworth[1]), adult birds (Young & Dreyer) and probably the chick embryo (Needham[4]) there is a cycle of base in its excretion; it passes through the excretory cells in salt form and later the cations are reabsorbed. Urea and ammonia are of course far more soluble and diffusible (solubilities in gm. N/100 gm. water = ammonia 47·0, urea 37·0, uric acid 0·0019).

On these views, as has already been said, the mammalian embryo is an aquatic

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[*] It must be understood that we are speaking here only of uricotelic excretion, i.e. the excretion of uric acid as the main end-product of nitrogen metabolism. Uric acid as the end-product of nuclein metabolism is an entirely different phenomenon, quantitatively much less important. Nuclein breakdown may not always go so far as uric acid; thus according to Truszkowski it stops at xanthine in *Hirudo* and *Anodonta*. The mammals have not, of course, lost the power of purine synthesis, in so far as they can construct their own nuclein (cf. p. 635 and Terroine & Mourot; Terroine, Giaja & Bayle).

[†] H. W. Smith (1932, p. 16; 1936, p. 74) is reluctant to accept the view that uricotelism and terrestrial oviparity are related, but he consistently avoids the difficulty that no other generalisation will explain the lack of uricotelism among the mammals, a plainly terrestrial class. It may be granted that the toxicity of urea has been somewhat overrated in the past, but the impossibility of the alternative of urea excretion need not have been the sole factor leading to uricotelism. A correlation has been suggested between selachian urea retention and cleidoicity (Needham & Needham in 1930; H. W. Smith in 1936). The only class of animals lower than the reptiles, it has been said, which have evolved a structure resembling the cleidoic egg are precisely the elasmobranchs. This may be viewed as an adaptation enabling the embryo to retain its maternal and its own urea in preparation for its adult marine life. Or, alternatively, it may be said that the survival value afforded by the protection of cleidoic egg-envelopes was only a possibility in animals for which the dispersal of nitrogenous end-products during development was, for other reasons, no problem. But this correlation is rather unconvincing, since some invertebrates, such as the cephalopods, have elaborate egg-cases and yet show neither urea retention nor uricotelism. Several authors, e.g. E. Starkenstein, have been keenly aware of the puzzling problems presented by uricotelism in relation to the mammals, but no one has proposed an alternative explanation which fits all the facts.

[‡] Confirmation of this is abundant, as in the work of Lvov & Roukhelman; Lawrie; Doyle & Harding, and others on various protozoa; Braconnier-Fayemendy on the leech, *Hirudo medicinalis*; Spitzer on numerous molluscs; and Mollitor on the crab *Eriocheir sinensis*. Teleostean nitrogen excretion is mainly as ammonia, selachian as urea; hence Manderscheid's failure to find the ornithine cycle in the liver of the trout was to be expected.

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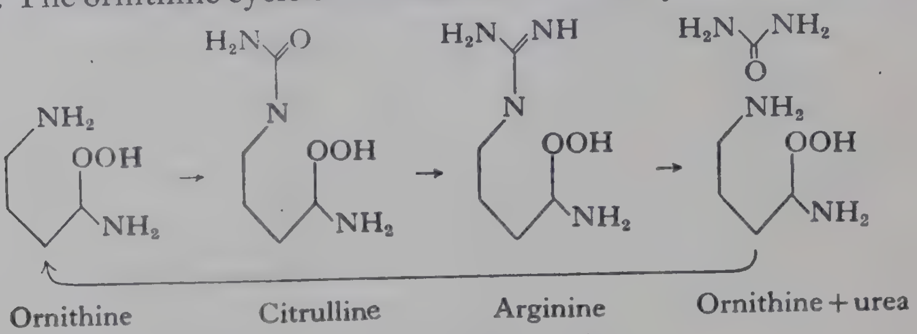
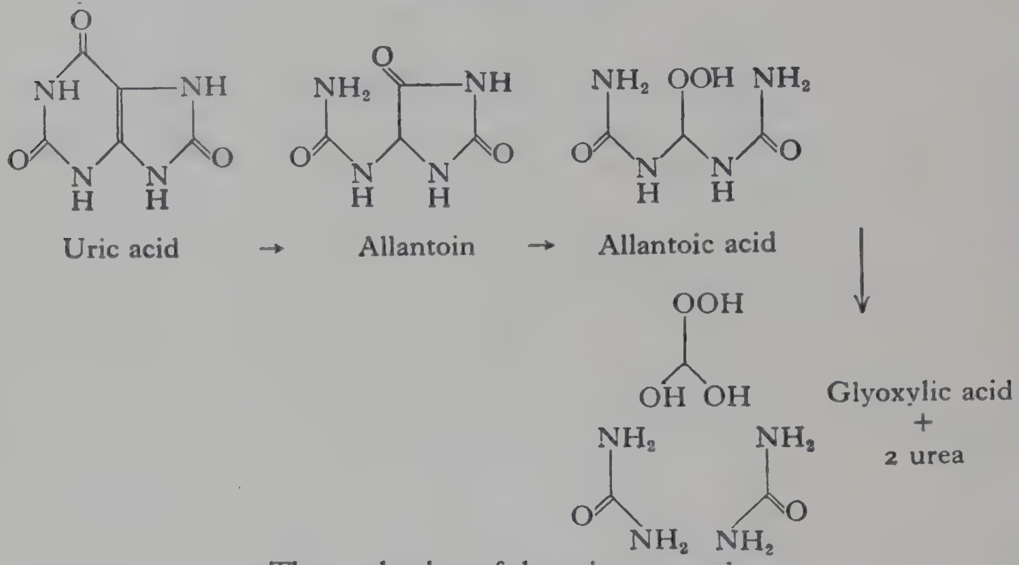
organism, its metabolism being adjusted to the unlimited water-intake of viviparity and to the easy removal of waste-products (by means of the maternal alimentary canal and the placenta and maternal kidney respectively). It is therefore not surprising that mammals are urea-producing organisms. And when we come to consider the phylogenetic relationships involved, we find they can be represented diagrammatically in a scheme somewhat similar to that shown in Fig. 18. The ornithine cycle of Krebs & Henseleit, by means of which urea is made from catabolic ammonia nitrogen in the mammals, reaches back, we know, into the realm of the fishes and amphibia (Manderscheid);* and it is therefore extremely probable that this was the manner in which urea was formed by the primitive pre-reptiles or stegocephalic amphibia from which modern mammals and modern sauropsida are alike derived. There were then at least four possibilities open: (1) to take on a terrestrial life, but by adopting true viviparity to retain the ancient system of urea production; this was the course taken by the Therapsids (Broom) and led to the mammals of the present day; (2) to take on a terrestrial life and to make use of the completely cleidoic egg, correspondingly passing to uricotelic metabolism, as did the sauropsida; (3) to retain a semi-aquatic mode of existence and to continue to lay non-cleidoic eggs outside the



The ornithine cycle.



The mechanism of the uricase complex.

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* But not into the invertebrates, where urea is not formed by the ornithine cycle, either in the snail (Baldwin & J. Needham) or the earthworm (Heidermanns[2]). We do not know whether all parts of the cycle are missing in the invertebrates. It may be significant that arginine desimidase (the enzyme producing citrullin, CO_2 and NH_3 from arginine) is absent in the hepatopancreas of *Astacus* (Ackermann).

ody; this led to the modern chelonia; (4) to burn no protein at all during mbryonic life and hence to avoid the problem of incombustible toxic nitrogenous esidues. This course alone would have permitted the existence of the terrestrial

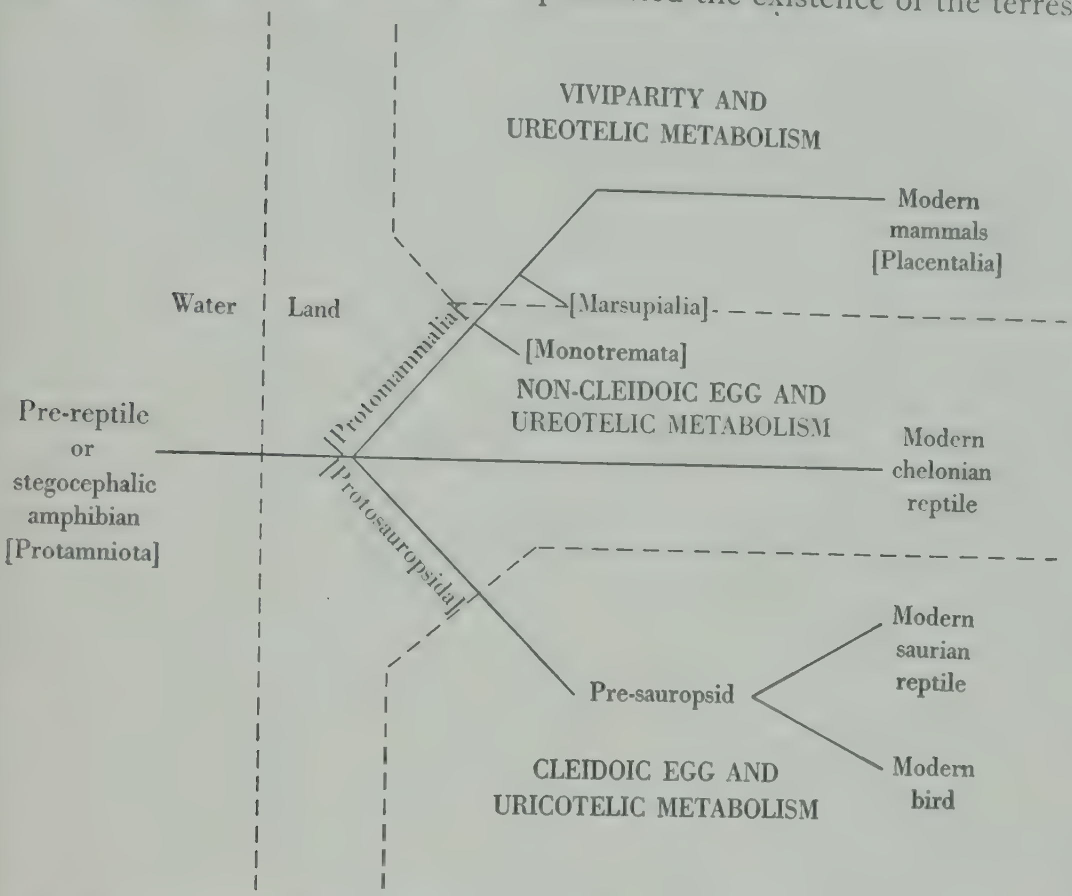
VIVIPARITY AND
UREOTELIC METABOLISM
Modern mammals [Placentalia]
Water
Land
[Marsupialia]
[Monotremata]
[Protomammalia]
NON-CLEIDOIC EGG AND
UREOTELIC METABOLISM
Pre-reptile or stegocephalic amphibian [Protamniota]
Modern chelonian reptile
[Protosauropsida]
Modern saurian reptile
Pre-sauropsid
CLEIDOIC EGG AND
URICOTELIC METABOLISM
Modern bird

Fig. 18. Scheme to illustrate the origins of viviparity and uricotelic metabolism.

. very similar scheme was arrived at independently by Portmann[3]. He remarks that it contradicts ıe common statement that the mammalian egg has undergone a secondary diminution of yolk, eing "derived from" a large egg of meroblastic sauropsid type.

H. W. Smith (1939, p. 62) advances another scheme. He suggests that by assuming warm-loodedness the earliest mammals were committed to filtering off a copious glomerular urine rom the blood because of their higher blood pressure, and hence to absorbing 90 % of it as man ow does. Hence they had less need of uricotelism. But would it not have given them an dditional advantage over the reptiles? If urea formation has no connection with oviparity and iviparity, why should not the birds, when they became warm-blooded and had to reabsorb water n their tubules, have reverted to urea formation? Evolution is irreversible only as regards regainments, not losses (cf. Needham[26, 31]).

leidoic egg without uricotelic metabolism, but so far as our knowledge goes to-day, life without protein combustion is impossible. The three courses actually taken are illustrated in Fig. 18.*

The position may be stated in another way by referring to Przylęcki's rule[1] (1926), which observes that in the animal kingdom uricase (the enzyme which breaks down uric acid) and uricoligase (the enzyme complex which forms it from catabolic nitrogen) are never found together. This is an important support for the view already expressed, that uricotelic metabolism is essentially an adaptation.

* On the information at present available to us, the acquisition of uricotelic metabolism must have occurred three times in evolution, once in the case of terrestrial molluscs, once in the case of terrestrial arthropods, and once in the case of the sauropsida.

That it is an adaptation to terrestrial oviparity follows from other considerations. It is of particular interest that during the first week of the chick embryo's development, an active uricase is present (Przyłęcki & Rogalski), but this soon disappears and the characteristic uric acid production supervenes. It is difficult to avoid seeing in this a phenomenon of recapitulatory significance,* since uricase is present in all fishes and amphibia, transforming the uric acid of their purine catabolism into glyoxylic acid and urea (work of several earlier authors, firmly established by Stransky in 1933). Some ammonia and urea is also formed during the chick embryo's development (cf. *CE*, Fig. 324), and here also a recapitulatory significance is likely, since ammonia is the typical excretory product of many invertebrates and of teleostean fishes, while urea is, as we have seen, characteristic of amphibia. But Needham, Brachet & Brown showed that the urea formed by the chick embryo is not a recapitulation of the amphibian condition, where urea is formed from the waste nitrogen of protein catabolism by the ornithine cycle; it is rather a recapitulation of the invertebrate situation, where urea is formed solely from arginase acting on arginine (as in *Helix*, Baldwin & J. Needham).

As Truszkowski & Goldmanówna, following others, have shown, uricase exists also in the hepatopancreas of many invertebrates, such as *Astacus* and *Anodonta*. "Uricase" is really three enzymes, uricase properly so called, which forms allantoin from uric acid, allantoinase, which converts allantoin into allantoic acid, and allantoicase, which breaks down the latter to urea and glyoxylic acid. Selachian uric acid breakdown, as Brunel has shown, comprises the whole chain of processes. In some teleosts (Brunel) the chain is also complete (e.g. *Esox*, *Cyprinus*, *Scomber*) but in others the last enzyme is missing and the breakdown stops at allantoic acid (e.g. *Salmo*, *Pleuronectes*, *Anguilla*). In amphibia the chain is again complete (Brunel), but in mammals, of course, the breakdown only goes as far as allantoin, with the exception of certain primates and the Dalmatian breed of dog, in which it does not occur at all. Lack of space forbids a treatment of this subject, one of the most interesting direct links between biochemistry and genetics (cf. Onslow; Rheinberger; Starkenstein; Young, Conway & Crandall; Trimble & Keeler).

Returning to Fig. 18, the modern Chelonia stand in an interesting intermediate position. It is known that alone among the reptiles they may show a considerable excretion of urea (cf. *CE*, Table 163, where urea excretion in turtles and tortoises is seen to be variable and high, in contrast to the saurian reptiles, where there is an overwhelming preponderance of uric acid). A recent finding illustrating the latter point is that of Benedict, who found 82 % of the solid urine of *Python* was uric acid, and in addition about 30 % of the nitrogen of the liquid urine. The difference between chelonia and other reptiles has been recently verified by Kuhl, and in conformity with this, Manderscheid found the ornithine cycle in the liver of *Testudo graeca*.† She noticed, however, that in the absence of ornithine, added ictate inhibited urea production, as if ammonia and lactate were forming some ther compound; a hint that in the land chelonia both urea formation and uric cid formation occur in the same organ. This has since been shown to be the ase by Heidermanns[3] and Münzel, who were the first to study chelonia of aquatic nd terrestrial habit side by side. Thus the urine of the semi-terrestrial *Testudo* ontains relatively much uric acid, while that of the semi-aquatic *Emys* contains elatively little. Now it seems that the course taken by the catabolic nitrogen in he chelonian liver varies with the temperature, for while at 20° much urea is ormed by *Testudo* liver-slices, at 37° the uric acid formation is double the urea ormation. There may be oecological significance in this, since terrestrial conlitions would generally be warmer than aquatic ones, but for the proper interpretation of the situation in the chelonia we need to know a good deal more about he nitrogen metabolism of their embryos. As we have seen (p. 35), their eggs ire non-cleidoic as regards water, but they may be, and probably are, cleidoic is regards the exit of nitrogenous waste-products.

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* It would be interesting to investigate other embryos from this point of view. There is a short note by Beck & Truszkowski stating that no uricase is to be found in early human embryos.

† Clementi[2], again, has recently shown that on autolysis urea is formed by the livers of mammals, amphibia, fishes *and* chelonia (*Testudo*); not by birds and saurians (*Lacerta* and *Tropidonotus*).

It will be remembered however that in one marine turtle Tomita[1] showed (*CE*,). 1103) that the predominant form of nitrogen excreted during embryonic life is irea. There must therefore here be a way out for diffusible substances as well is a way in for water. Imamura's analyses[2] of the allantoic liquid of this animal ire too fragmentary to help us much.

In the liver of *Testudo* the urea production does not fall off as the uric acid formation rises to its maximum, as it would do were urea an intermediate stage in the production of uric acid. On the contrary, it rises to a maximum of its own at a lower level. This adds further support to the conclusion (already well established by the work of Clementi[1] and his school, to which references will be found in Needham, Brachet & Brown) that the uric acid synthesis of the sauropsida does ot go by way of urea. Its exact nature is still uncertain, but it was shown ndependently by Schuler & Reindel and by Benzinger & Krebs that in pigeons he collaboration of two organs is necessary for uric acid formation, the liver ynthesising from ammonia and carbon atoms a precursor substance which the idney converts into uric acid. In the hen and the goose both processes can go n in the liver. Edson, Krebs & Model later showed that the precursor substance passing to the kidney is hypoxanthine. Its conversion to uric acid is accomplished by xanthine oxidase and does not need the cell-integrity of the kidney tissue, but he synthesis of hypoxanthine in the liver does need the normal cell structure and hence will only proceed in tissue-slices. The mechanism of this synthesis is still quite obscure, but Örström, Örström & Krebs have shown that it is specifically accelerated by oxaloacetate, which perhaps provides the carbon chain, and by glutamine, which perhaps provides the ammonia. Glutamine may therefore be an important intermediate substance. Örström, Örström, Krebs & Eggleston find that of ammonia given directly to liver tissue 50 % is converted to glutamine and 20 % directly to hypoxanthine.

The Prototheria (Monotremes), like the chelonia, stand in an intermediate position (see Fig. 18). As already pointed out (*CE*, p. 1145) *Echidna* has a typically mammalian urinary nitrogen-partition (and this has since been confirmed by

Mitchell), but it is marsupial and the pouch must act as a uterus more successfully than it does in *Hippocampus* (see p. 47), where the eggs are very yolky. Here the finding of Hill & Hill is significant; the shell of the marsupial monotreme egg has only the basal layer and no calcareous accumulation, as in *Ornithorhyncus*. What we need to know is the urinary nitrogen-partition of the non-marsupial monotreme, *Ornithorhyncus*, and this the literature does not divulge.

We come now to terrestrial oviparity and the cleidoic state among the invertebrates. It should be associated with uricotelic metabolism, and so it is, as the two important groups of insects[*] and terrestrial gastropod molluscs testify.

That the nitrogen excretion of insects consists almost wholly of uric acid has been noted again and again.[†] Uricotelic metabolism begins already at the "link" between insects and annelids, as we know from Manton & Heatley's work on *Peripatopsis*. But just as we have seen that the chick embryo in its early stages excretes a little ammonia and urea, so too the aquatic larvae of insects excrete ammonia (e.g. the may-fly larva, Fox and S. Smith[2]). Ammonia excretion was early reported for the larvae of muscid flies, but this was disregarded owing to the enormous quantities of bacteria in their guts, until Hobson (confirmed by Michelbacher, Hoskins & Herms and by Lennox) demonstrated that even when fly larvae were raised in scrupulously sterile conditions there was a considerable ammonia production.[‡] The phenomenon was studied in detail by Brown[1] on *Calliphora erythrocephala* and *Wohlfahrtia vigil*; he found that the uric acid excretion was only $\frac{1}{20}$th that of the ammonia, but that both rose to a peak corresponding to the growth maximum and then fell to zero at the onset of pupation. During pupation uric acid alone is formed (cf. the discussion of Metamorphosis on p. 470, where it is shown that the metabolism of pupae has cleidoic characteristics). Correspondingly, Truszkowski & Chajkinówna found uricase in the housefly and the blowfly (*Musca domestica* and *carnaria*). Somewhat puzzled, they concluded that Przyłęcki's rule does not hold for the insects, but the subject was restored to rationality by Brown[3], who was able to show that in the fleshfly or sheep blowfly, *Lucilia sericata* (sterile), the uricase present in the larvae suddenly disappears at the beginning of pupation, and as suddenly appears again when the adult emerges from the pupal state. In *Lucilia* the following figures hold good:

	Ammonia	Uric acid	Allantoin
Embryonic period, mgm./gm.	0·009	0·178	0·106
Larval period, mgm./100 individuals	41	0·5	2·5
Pupal period, mgm./100 individuals	2	27	3
Adult period, 1st week of life, mgm./100 individuals	1	8	2

---

[*] We ought perhaps to add the arachnids with their guanine excretion, for it is practically certain that this is a special type of "uricotelic" metabolism. The nitrogen excretion of all arthropods has been well reviewed by Maluf[3].

[†] Newly quantitatively confirmed by Heller & Aremówna on the hawk-moth, *Deilephila euphorbiae*; Leifert on the moth, *Antheraea pernyi*; Brown[2] on the grasshopper, *Melanoplus bivittatus*; Truszkowski & Chajkinówna on the black-beetle, *Blatta orientalis*, the cockroach, *Blatella germanica*, and the may-beetle, *Melolontha vulgaris*; Becker[2] on the hornet, *Vespa crabro*, and the yellow butterfly, *Gonepteryx rhamni*; Wigglesworth[1] on the blood-sucking bug, *Rhodnius prolixus*; etc. etc. Becker points out that the pterin pigments of insect wings (see p. 655), though purine derivatives, never occur in insect excreta.

[‡] The larvae, according to Tomita & Kumon, contain urease.

Thus during embryonic life about twenty times as much uric acid is produced as ammonia, while during larval life about eighty times more ammonia is produced than urea. The pupa reverts to the embryonic relationship, and the adult to the larval. Brown remarked that the semi-aquatic mode of life of muscid larvae would eliminate the need of an excretory product adapted for terrestrial existence and thereby render available the energy yielded by oxidising uric acid to allantoin.[*] Similarly, the existence of uricase in the adult may be regarded as a secondary adaptation making the excreta of other animals available to the insect as sources of energy. Rocco's report of uricase in dytiscids and some orthoptera, and of allantoinase in carabids, requires confirmation.

About the mechanism of uric acid synthesis in insects no information is available except from the work of Leifert, who studied it in the moth *Antheraea pernyi*, but as the experiments were made on breis in the doubtful absence of bacteria, her conclusion that urea is an intermediate must be regarded as only provisional.[†]

A few words must now be said regarding the terrestrial molluscs. Strohl[1], in his valuable review of the excretory mechanisms of molluscs, came to the general conclusion in 1914 that the gastropods are characterised by positive findings of uric acid in their nephridia, but that in the lamellibranchs this substance is always absent, and its place taken by urea. This is obviously in rough agreement with all that has so far been said, seeing that the former embody the large class of terrestrial pulmonates and that the latter are wholly aquatic. But this generalisation rested only on qualitative tests scattered through the literature, unsatisfactory because a positive uric acid test might imply no more than the presence of a nuclein catabolism of the usual type and nothing about the presence of uricotelic metabolism. It remained for later investigators (Wolf; Baldwin & J. Needham; Baldwin[2]; Grah) to demonstrate with varying degrees of success by *in vitro* experiments that the snail does actually form uric acid from added ammonia or amino-acids.[‡]

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[*] The beneficial effects of maggots in the healing of certain wounds are to be ascribed partly to the allantoin and urea which they excrete (Robinson; Brown[2]) and partly to the sterilising effect of the alkaline *p*H caused by their ammonia excretion. Brown & Farber have studied an interesting anaerobic deaminase in fleshfly larvae which acts only on polypeptides and not on any simpler protein breakdown-products.

[†] A part of the work of the Bonn school seems to me to be vitiated by the use of enormous amounts of precursor substance relatively to the end-products estimated. Thus a rise from 1 mgm. to 2 mgm. of the end-product after an hour's incubation with 500 mgm. of the precursor in the presence of the tissue is claimed as a 100 % increase. One wonders whether a stricter statistical treatment is not necessary in the case of these heterogeneous breis.

[‡] It seems rather unlikely that the course of synthesis is the same in birds and gastropods, because Baldwin & J. Needham found nothing in the snail hepatopancreas which the pigeon kidney could convert into uric acid, and observed that the snail hepatopancreas could not make uric acid from the products of the pigeon liver. For the reasons given in the preceding footnote, Wolf's contention that urea is an intermediate in uric acid synthesis in *Helix* cannot be accepted; the similar result of Baldwin[2] is slightly more convincing because the quantities of precursor added were of the same order as the syntheses obtained. The question, however, is by no means closed. As in birds, the integrity of cell structure is necessary for uric acid synthesis by the gastropod hepatopancreas (Grah). Arginase may be connected with it, for Baldwin[1] has found very large amounts of this enzyme in the uricotelic gastropods and none in the others.

That the terrestrial pulmonate gastropods were really uricotelic, however, had long been probable by reason of the extraordinary accumulations of uric acid in their nephridia. Quantitative estimations give figures such as 660, 720 or 810 mgm. per gm. dry wt. for the nephridium of the edible or vineyard snail, *Helix pomatia*, so that no less than three-quarters of the dry weight of the organ may consist of uric acid. Starting from this point a comparative survey of the uric acid retentions of many gastropods was made by Needham[18] in the hope that the presence of uricotelic metabolism might reveal itself by high uric acid figures.* It must be emphasised that in the absence of information regarding the speed of excretion in each of the species studied, the results cannot be made the basis of any final scheme, but there are reasons for believing that the excretory rate of all molluscs is very slow. The data are worth examination, because like those obtained for the selachian fishes, they illustrate a wide range of oecological, physiological and biochemical differences all within one group of animals (see Fig. 19).

The general results were clear. Beginning with eight species of marine gastropods (operculates) occupying the primitive aquatic environment, it was found that in all cases their uric-acid content was very low† (N. 2–$4\frac{1}{2}$, W.B. 0·2–0·5). This was no more than might be expected from nuclein catabolism. On the other hand, we may contrast with these, as the opposite extreme, the fully adapted terrestrial gastropods—pulmonates such as the English *Helix* and *Helicella* or the Paraguayan *Bulimulus*; or the operculate *Cyclostoma*. Here the retentions were found to be extremely high, ranging from N. 150, W.B. 4 in the case of *Helicella* to N. 700, W.B. 10 in the case of *Helix pomatia* and N. 1000, W.B. 80 in the case of *Cyclostoma*. For these figures it seems quite impossible that nuclein catabolism could be responsible, even if no excretion of uric acid ever took place throughout the organism's life cycle; uricotelic metabolism must be present. It is sometimes supposed that these terrestrial gastropods made their way on to the land by direct littoral penetration; if so, the modern series of periwinkles shows the process in action. Comparative estimations of uric acid in the Littorinidae showed that whereas the most marine of them (*L. littorea*) occupies a position quite analogous to the marine operculates (N. 1·5, W.B. 0·1), the most terrestrial of them (*L. neritoides*) approaches the terrestrial level (W.B. 2·4).

The data on the gastropods of fresh-water were rather complicated and offered difficulties of interpretation discussed elsewhere (Needham[31]). Some of the fresh-water pulmonates, such as *Limnaea stagnalis*, always regarded as secondarily inhabiting fresh-water, agreed closely with the land pulmonates (N. 100, W.B. 3), and Baldwin[3] has shown that their embryos, like those of *Helix*, accumulate uric acid as they develop. On the other hand, there are cases, such as *Planorbis* or *Ancylastrum*, where the nephridial uric acid is very low. These it is easy to explain as due to a more vigorous flow of water through their bodies, but such an explanation will not meet the interesting cases of *Paludina* (*Viviparus*) or *Bithynia*, esh-water operculates which, owing to their gills and lack of mantle vascularisa-on, must surely have reached their present position by colonisation up the rivers. or these operculates contain considerable amounts of uric acid in their nephridia N. 35, W.B. 5). Possibly they originated not directly from the sea, but by way f a period of semi-terrestrial or terrestrial life, analogous to that of *Cyclostoma*, ith its vestigial gill, and so acquired uricotelic metabolism.

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* The subsequent work of Spitzer went independently over part of the same ground, with substantially the same results.

† Abbreviations: N. = nephridium; W.B. = whole body; figures in mgm. uric acid per gm. dry wt.

It is pertinent to enquire in this connection how far the eggs of terrestrial astropods are truly cleidoic. Not much is known about them. The observation f Baldwin[3], that *Limnaea* eggs accumulate uric acid, has just been mentioned,

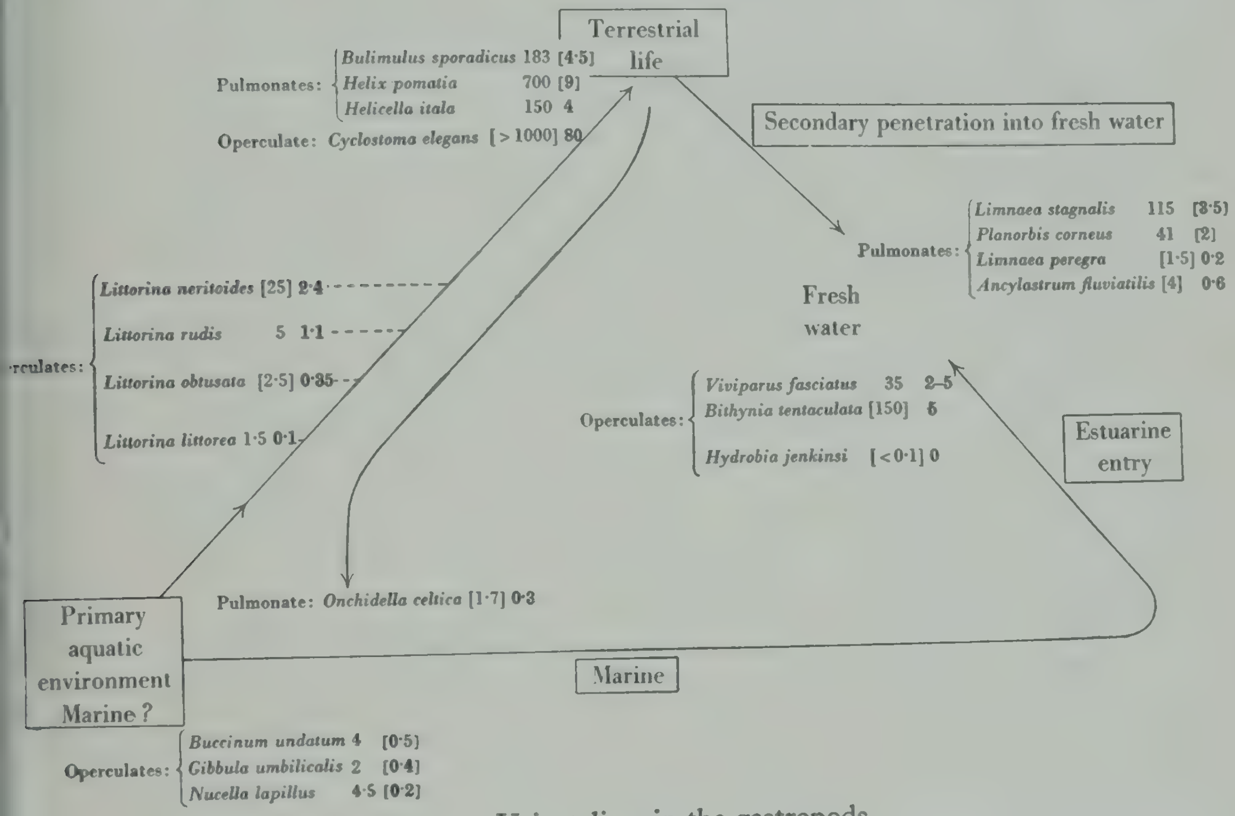
Terrestrial life
Pulmonates: Bulimulus sporadicus 183 [4·5]
Helix pomatia 700 [9]
Helicella itala 150 4
Operculate: Cyclostoma elegans [> 1000] 80
Secondary penetration into fresh water
Pulmonates: Limnaea stagnalis 115 [3·5]
Planorbis corneus 41 [2]
Limnaea peregra [1·5] 0·2
Ancylastrum fluviatilis [4] 0·6
Fresh water
rculates: Littorina neritoides [25] 2·4
Littorina rudis 5 1·1
Littorina obtusata [2·5] 0·35
Littorina littorea 1·5 0·1
Operculates: Viviparus fasciatus 35 2–5
Bithynia tentaculata [150] 5
Hydrobia jenkinsi [< 0·1] 0
Estuarine entry
Pulmonate: Onchidella celtica [1·7] 0·3
Primary aquatic environment Marine ?
Marine
Operculates: Buccinum undatum 4 [0·5]
Gibbula umbilicalis 2 [0·4]
Nucella lapillus 4·5 [0·2]

Fig. 19. Uricotelism in the gastropods.

Nephridium figures in ordinary type, whole body figures in black type. Unbracketed figures are experimental, bracketed are calculated; see Needham[31].

nd a comparison may be made between the eggs of *Helix* and of the hen. The former accumulate 0·3 mgm. % of the final dry weight in uric acid by he time of hatching; the latter some 0·7 % – values of the same order. The snail embryo loses 19 % of the dry weight of the egg by combustion; the chick embryo 18 %. On the other hand, the snail embryo loses 48 % of the wet weight of the egg by evaporation as against the 15 % similar loss by the chick embryo. But Carmichael & Rivers have shown that the snail embryo will still develop normally after a dehydration of the eggs amounting to 85 %. There is certainly no evidence that the snail egg absorbs water or anything else but oxygen from the environment, and it may therefore be that the snail, instead of providing the relatively impermeable shell of the bird's egg, provides a much greater excess of water in its eggs above the embryo's minimum requirements.

Lastly, it may be mentioned that the presence of uricase (contrary to Przyłęcki's rule) in the gastropod hepatopancreas has been claimed by some workers (Spitzer; Grah; Plum). Of these the only convincing paper is that by Plum who isolated allantoin from hepatopancreas, but as the evidence even in this case depends upon autolysing breis we have no guarantee that the uricase ever functions as such in the intact gastropod, and every likelihood to the contrary.

All that has so far been said does not exclude the possibility that ovoviviparity and even viviparity might arise late in evolution after the uricotelic habit had become so fixed that it could no longer be lost. It seems that this has occurred in all the terrestrial and uricotelic groups which we have been mentioning. Thus, to take the gastropods first, the two most terrestrial species of periwinkles (*L. rudis* and *neritoides*) are ovoviviparous (Lebour), while the two others are not. *Paludina* is a classical case of ovoviviparity. We may probably assume, therefore, that gastropod ovoviviparity involves merely protection and no exchange of organic substance between the maternal organism and the eggs—a statement which must remain tentative until some exact studies are made of gastropod ovoviviparity from the present standpoint.* Among insects,† too, there are instances of larviparity and even pupiparity, but except in the rarest cases, such as the polyctinid‡ bug, *Hesperocterus fumarius*, studied by Hagan, there is no attempt at a placenta. The insect types may be classified as follows (Hagan; Keilin¹):

Ovoviviparity. The egg contains enough yolk to carry the embryo to hatching; after this the larva is extruded by the maternal organism without receiving further nutriment (Coccidae, Coleoptera, Sarcophagidae, etc.).

Intussuctio-viviparity. The egg contains enough yolk to carry the embryo to hatching; after this the larva is nourished in the uterus by specialised organs (Diptera).

Exgenito-viviparity. The embryo, in a stage of development corresponding to the egg stage of ovoviviparous forms, obtains nourishment by means of a trophamnion, trophoserosa, or trophochorion. Development occurs in the haemocoele, not in the uterus (Strepsiptera).

Pseudo-placento-viviparity. A placenta-like organ is present. Only two cases known: *Hemimerus* (Dermaptera) and *Hesperocterus* (Hemiptera).

It must be emphasised that all these cases are exceptional, but biochemical studies of their nitrogen excretion would be curious and interesting.

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* It is true that according to Litwer, the gill epithelium which helps to form the brood-pouch in sphaerid lamellibranchs, has histologically a secretory appearance. For details of their development, see K. Okada.

† It used to be thought that the embryos in the brood-pouch of *Daphnia* were nourished by the maternal organism, but this crustacean ovoviviparity has been disproved by Rammer. Ovoviviparity is common among crustacea, especially cladocera and isopods. Nevertheless Gravier's review of crustacean ovoviviparity shows that structures suspiciously like secretory organs occur in some crustacean brood-pouches, e.g. *Polyphemus*. These questions do not affect the issue of nitrogen metabolism, except as regards land-crabs, which deserve a study they have never received.

‡ For an account of this strange and rare group see Ferris & Usinger.

When we turn to the vertebrates, we find that though there are no ovovivi- rous birds, ovoviviparous or viviparous reptiles are by no means unknown (cf. : reviews of Wunder; Graham-Kerr, etc.). Giacomini discovered a kind of iparity in the Italian lizard *Seps chalcides*, and Flynn und it to be widespread among Australian reptiles. ie detailed descriptions of Tencatehoedemaker for *ps* (see Fig. 20) and of Weekes for the Australian ards *Egernia cunninghami*, *Tiliqua scincoides* and *gosoma* spp., among others, indicate that the contact tween maternal and foetal tissues is, in all cases, like at of the placental dogfish, more or less epithelio- iorial. Certain Australian snakes, however, such as *enisonia superba* (Weekes), seem to have a more timate connection between maternal and foetal ssues than this. It is lamentable that no information out the nitrogen metabolism of such forms exists, it we may predict that it is uricotelic, and that the viparity is of late evolutionary origin.*

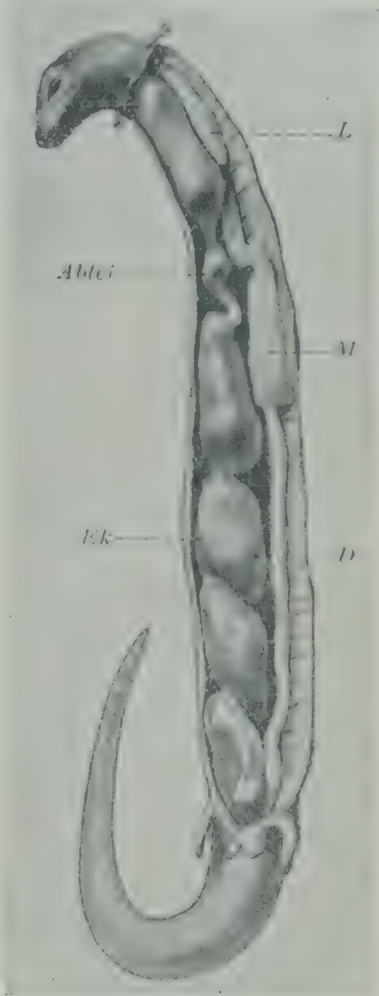

Fig. 20. Pregnant female of the lizard *Seps chalcides*. *Abtei*, abortive embryo; *Ek*, embryo compartment; *D*, intestine; *M*, stomach; *L*, lung.

The course of this discussion may be summarised ; follows. The only guiding thread† through the ewildering mass of facts in the comparative physio- gy and biochemistry of nitrogen excretion seems to e the conditions under which the embryos of the pecies have to live. We may formulate it in the atement that uricotelic metabolism and terrestrial viparity are strictly correlated, and this is perhaps e most remarkable instance of the far-reaching etabolic effects of a particular embryonic mode of e. It is not, of course, the whole of the story, r a number of minor specialisations such as the cretion of guanine or trimethylamine exist, and oviviparity or even viviparity may in rare instances pervene upon uricotelic metabolism after this has come so fixed that its loss is impossible. Nevertheless, the correlation in neral abundantly justifies itself.

We will now turn for a moment to the last of the metabolic properties of the cidoic state, namely the preponderating reliance on fat catabolism (cf. Table 10). s already suggested (*CE*, p. 1186), the provision of metabolic water (the copious ydrogen in the paraffin chain gives about an equivalent weight of water for every itty acid molecule burned) may be connected with this, where water needs are

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This view has the approval of one of the authorities on this subject, T. T. Flynn, expressed to e author in a private conversation.

It is important in this subject not to be led astray by false trails. Thus, for his statement that icotelic metabolism may exist in marine copepods, Maluf ([3], p. 52) relies on a statement made 1858, and Heidermanns ([1], p. 266) has nothing later than 1872 in favour of the occurrence of uric id excretion in ascidians.

great, as in terrestrial eggs. Allied with it is the property of unusually hi resistance to ketosis (Needham[13]).

From the data given in *CE*, pp. 920 ff., the diet of the chick embryo each d during its development can easily be calculated. This is shown in Fig. 21, terms of carbohydrate, fat and protein in percentage of the total material absorbe from which it will be evident that by far the largest proportion of what is absorb by the embryo is fat and protein. At certain stages, indeed (about the 10th a 19th days), twice as much fat as protein is absorbed. Moreover, as we shall s (p. 592), the material combusted during the last half of development seems be almost entirely fat.

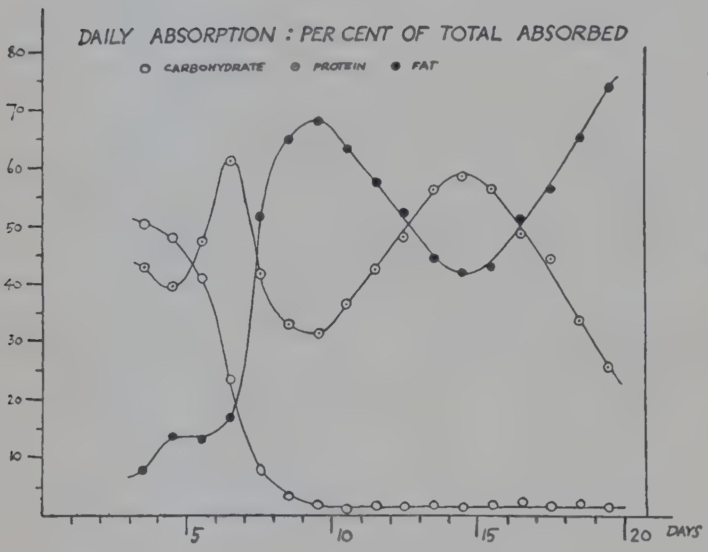

Fig. 21. Daily absorption of foodstuffs by the chick embryo.

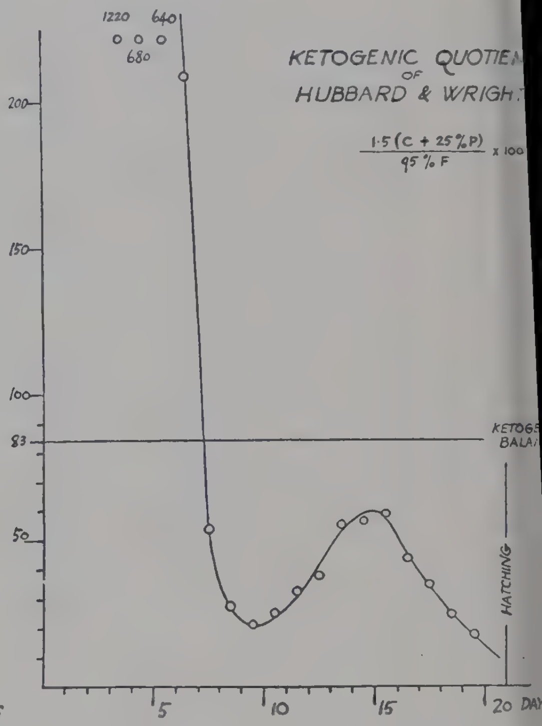

Fig. 22. Ketogenic quotient of the chick embryo.

Now the excretion of aceto-acetic acid and β-hydroxybutyric acid is associated as has long been known, with starvation, severe diabetes, and the restriction o carbohydrate intake. Of these three conditions, the third is eminently observabl in the "unbalanced" diet of the chick embryo, and if the ketogenic balance i calculated according to the method of Hubbard & Wright, as in Fig. 22, it ca be seen that from the 7th day of incubation onwards the chick embryo is belo the danger line. In spite of this fact, however, the chick embryo develop absolutely no ketosis; no keto-acids can be found in the allantoic liquid or elsewhere (Needham[13]), though K. Takahashi[1] detected minute traces of acetaldehyd and acetone. As a search of the literature fails to discover any case of ketosis i an adult bird, even in a diabetic state, it is possible that immunity to dietar ketosis may be another special adaptation to cleidoic development with its intensification of fat absorption and fat catabolism. At the same time, it is true tha certain mammals, such as the pig, and even certain types of human being, suc he Eskimo, have remarkable powers of resistance to dietary ketosis. Further rk along these lines would be desirable.

n the course of this section, which, in following a single line of argument, has ged over a wide variety of topics, three points have been raised which require ther treatment. These are (1) the normal curves of mortality in avian develop- nt, which may throw light on the special qualities of the cleidoic egg, (2) the irse of embryonic nutrition in birds, and (3) the biochemistry of the highest m of viviparity, the mammalian placental mechanism. These will be the jects of the following three sections, and with them we shall complete what s to be said about the nutritional substratum of morphogenesis.

7. Standard mortality curves

Payne was the first to make a detailed study of the points in normal embryonic velopment under optimum conditions at which the maximum mortality occurs, d he has been followed by numerous workers (e.g. Byerly[2]). The fact that under ch conditions there is always a certain loss of embryos indicates that the idoic egg of birds is not a 100 % efficient vehicle of reproduction. And the gitimate hope has been entertained that if we could find out the reason for these rticularly dangerous periods, we should gain a better understanding of the ture of embryonic metabolism in its cleidoic form.

It was early recognised that the two main mortality peaks occur at about 4 days and 18–19 days of incubation. Fig. 23 shows the mortality distribution the fowl embryo (*Gallus*) compared with that of the common pigeon (*Columba*), e ring dove (*Streptopelia*) and various wild Peristeridae (*Turtur*, *Spilopelia* and *noidura*). The mortality of the turkey, the ring-necked pheasant, and the b-white quail, are also given. In all cases, notwithstanding the rather different tal incubation times (14–28 days), the peaks occur at equivalent moments of embryonic life history. Riddle[3] has given the most probable explanations of peaks in saying that the first is connected with respiratory maladjustments and second with difficulties of water metabolism. "The method of avian repro- ction", he wrote, "requires that water be stored in such quantity as will still adequate at the end of incubation; but the very means—thick shells—which egg uses to restrict the rate of water-loss may, if carried too far, adversely strict the particular rate of gaseous exchange which is most favourable for the ly developmental stages." Hence mortality might be expected to be most vere at the beginning, before the establishment of the area vasculosa, the antois, etc., and at the end, when the water-supply, owing to continuous and avoidable evaporation, is running low.

In favour of the respiratory nature of the first peak there are many facts entioned in *CE*, p. 1417 etc.), such as the death of embryos in eggs accidentally tained in the oviduct, the marked sensitivity of embryos before the 4th day of cubation to abnormally high or low oxygen pressures, etc.

That the second peak is connected with water-loss is also fairly well established. or terrestrial embryos the supply of water is, as we have seen, an important oblem. The egg-white of reptiles and birds is the equivalent of the jelly rrounding amphibian and dipnoan eggs. The cleidoic egg was an obvious device prevent undue evaporation of water. It includes, moreover, means whereby e pressure-head of water for the embryo is kept at a relatively constant level, r during development the egg-white is acidified (cf. *CE*, p. 881) and so brought to its isoelectric point, allowing the liberation of water by degrees from colloidal albumen. The safety margin of water-loss in birds' eggs may be sm On the one hand, it is true that in Weldon's experiments the addition of w to the incubating egg, in such a manner as not to interfere with the proce of respiration while replacing that normally lost by evaporation, led to malforn tions of the amnion and the death of the embryo. But on the other hand Rid conclusively showed, using the eggs of tropical birds, that an increase of the nor loss of water by evaporation leads to the death of the embryo. It is impossibl

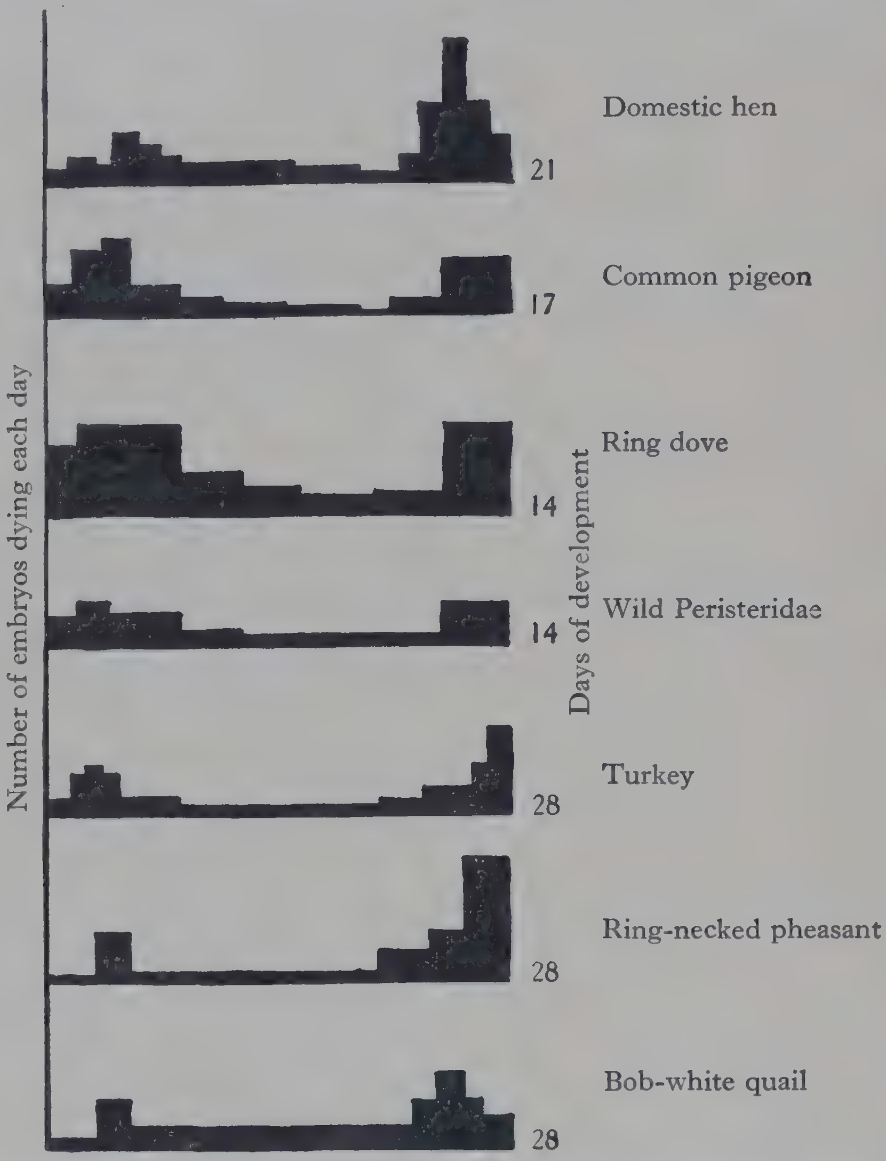

Fig. 23. Distribution polygons showing normal mortalities of seven kinds of birds (the first fo from Riddle, the fifth from Martin & Insko and the last two from Romanov).

hatch the eggs of pigeons from the climate of Venezuela (the Orinoco region) i the temperate moderately, dry air of North America, but if placed partly in wate they would develop normally and hatch. Death in the former circumstanc usually took place during the final two days of incubation and was accompanie by abnormally large air-sacs, indicating excessive evaporation. Indeed, Lippinco & du Puy were able to hatch hen's eggs successfully if they were incubated lyin in a layer of distilled water $\frac{1}{2}$ in. deep. Calculation shows that one hen's egg los by evaporation 9·8 gm. water out of an original 38·4 gm. and gains from th ɪmbustion of fat 2 gm. Hence the latter quota may be of importance (cf. above, 48 and p. 62).

It is doubtful, however, whether the standard mortality peaks are wholly to : accounted for by the above-mentioned causes. As we have seen (p. 22), they 'e affected by the diet of the laying hen (Byerly, Titus & Ellis; McFarlane, ulmer & Jukes), and they may also be affected by toxic substances introduced .rectly into the egg. When Landauer[4] introduced toxic doses of Mg″ and Li′ .lts into developing eggs on the 6th day of incubation there was no effect on the nbryonic death rate until the second standard mortality peak, when a much :eater mortality than usual took place. A slightly higher mortality of male than ·male embryos in the fowl is said to exist (Landauer & Landauer), but we do ot know at which of the two peaks this selective elimination takes place.

Temperature also has a slight effect upon the peaks. The optimum temperature ıd optimum humidity for the development of the chick embryo are now very :curately known (see the review of Landauer[23]; the papers of Romanov and his ɔllaborators; Penionskevitch & Retanov, etc.). The optimum temperature is not constant but varies with the humidity of the air; as the latter increases, the ɔrmer decreases. There is general agreement that the susceptibility of the chick mbryo to periods of chilling increases steadily and continuously through evelopment (Kaufman[7]; Taylor, Gunns & Moses). Romanov, Smith & Sullivan ave shown that the second mortality peak is affected by all kinds of variations f temperature away from the optimum.

Contributory to the peaks of mortality, though only to a small degree, are the arious "malpositions" which have been much studied in America (Byerly &)lsen; Olsen & Byerly; Waters; Hutt & Pilkey; Cavers & Hutt; Hutt & Cavers; Iutt & Greenwood; Dove). These are classified as follows:

I. Head between legs.
II. Head in small end of egg.
III. Beak under left wing.
IV. Head buried away from air-space.
V. Feet over head.
VI. Beak over right wing.

he first and third of these occur with especial frequency when the egg is cubated large end up; the second and fourth when it is incubated horizontally. he first is invariably lethal, the second probably so (50 %), the fourth is nearly ways lethal and is determined during the 2nd week of incubation. Olsen & yerly believe that the pointed end of the egg should be caudal, and if this lation fails to arise during the preparation of the egg, malpositions are likely to :sult. Waters thinks that malpositions are often semi-normal stages which most mbryos pass through but which in some cases are retained and so prevent hatching. 'he interesting phenomenon of *heterotaxia*, i.e. the position of the embryo lying n its right side instead of on its left, as normally, occurs to the extent of 0·163 % mbryos and has no effect on hatchability (L. W. Taylor). Malpositions have lso been studied in the turkey (Martin & Insko; Insko & Martin).

It would be very interesting to have data regarding the standard mortality urves of animals other than birds. Fragmentary data exist for certain fishes *CE*, p. 1385), and for the silkworm Grandori has classified three kinds of "dead-n-shell" embryos. There are those which have gone no further than the blasto-erm stage, there are others which have produced many tissues but in which no ıetameric segmentation has appeared, and still others which have produced bnormally formed organs. Unfortunately no statistical figures or times are vailable here. For a good discussion of foetal death in man the reviews of 'faundler and Sterling may be referred to. Rodents are more fortunate in that septic resorption of dead embryos following atrophy is carried out with remarkable facility (Crosman; Corey[2]), cf. *CE*, p. 1329, and this may even happ in cattle (C. W. Turner). As Kosaka has shown, foetal atrophy usually begi in the liver, which becomes progressively less prone to autolysis (as judged by t extent to which it has proceeded in a 72 hr. period) during development. It mu be a faculty widespread in the animal world, since Guareschi[3] observed it happenin in dead embryos in the modified ovary* of the teleostean fish *Gambusia*.†

1·8. Embryonic nutrition

1·81. The scaffolding of the chick embryo

The temporary structures used by the embryos of birds during their develo ment are three in number, the yolk-sac through which the absorption of nutrie materials from yolk and white is carried on, the amnion which encloses th embryo in its "private pond", and the allantois which receives the excreta fron the cloaca and allows the exchange of respiratory gases through its blood-vessel closely applied to the inner shell-membrane. We have no need to add to th excellent descriptions of them already available, such as that of Lillie[8], whos diagram is reproduced as Fig. 24; but as to their physiology there is a good dea to be said in amplification of previous accounts (*CE*, pp. 917 ff.).

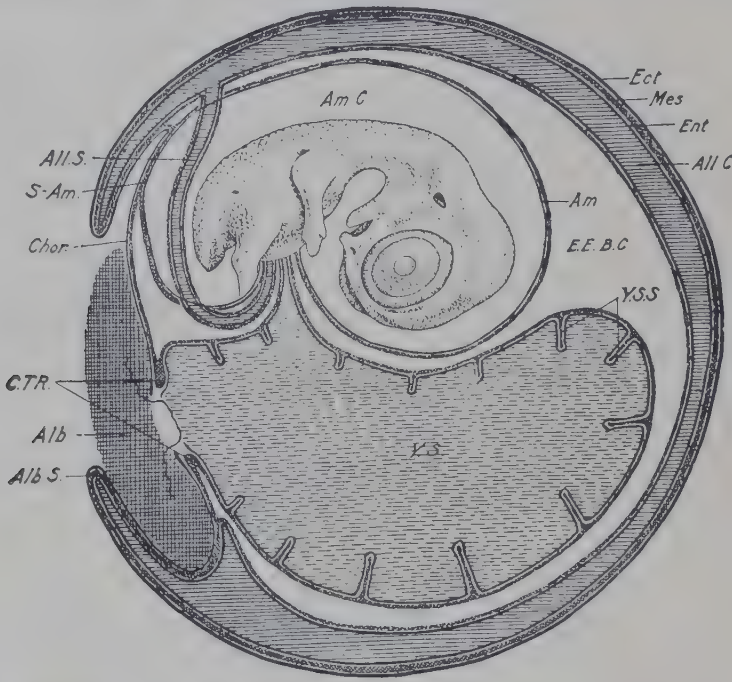

Fig. 24. The membranes of the chick embryo. *Alb.*, albumen; *Alb.S.*, albumen-sac; *All.C.*, allantoic cavity; *All.S.*, allantoic stalk; *Am.*, amnion; *Am.C.*, amniotic cavity; *Chor.*, ecto-mesodermal portion of extra-embryonic blastoderm associated neither with yolk-sac nor with allantois ("chorion"); *C.T.R.*, mesodermal ring surrounding a small area of the yolk which the blastoderm fails to enclose. The remains of the vitelline membrane can be seen; *Ect.*, ectoderm; *E.E.B.C.*, extra-embryonic "body-cavity"; *Ent.*, endoderm; *Mes.*, Mesoderm *S-Am.*, sero-amniotic connection, later perforated; *Y.S.*, yolk-sac; *Y.S.S.*, villi of the yolk-sac wall (see Fig. 28).

Byerly[4] was the first to study the membranes of the chick embryo quantitatively measuring wet and dry weight, and his treatment of the question remains th best. The yolk-sac grows steadily from the 1st day onward to reach a maximun et weight of about 3·5 gm. on the 15th day of incubation, after which its weight lls to approximately 2·5 gm. at hatching. The allantois does not appear until e 4th day, but it then grows more quickly than the yolk-sac to reach a maximum et weight of about 1·4 gm. by the 10th day, after which it remains roughly nstant in weight till the time of hatching. The third membrane, the amnion, s a much less bulk than either of the other two. Byerly's most striking chart ig. 25) demonstrates that not until half-way through development does the

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* See p. 46.

† But see p. 260, where the unshed unfertilised eggs seem to give rise to tumours in worms.

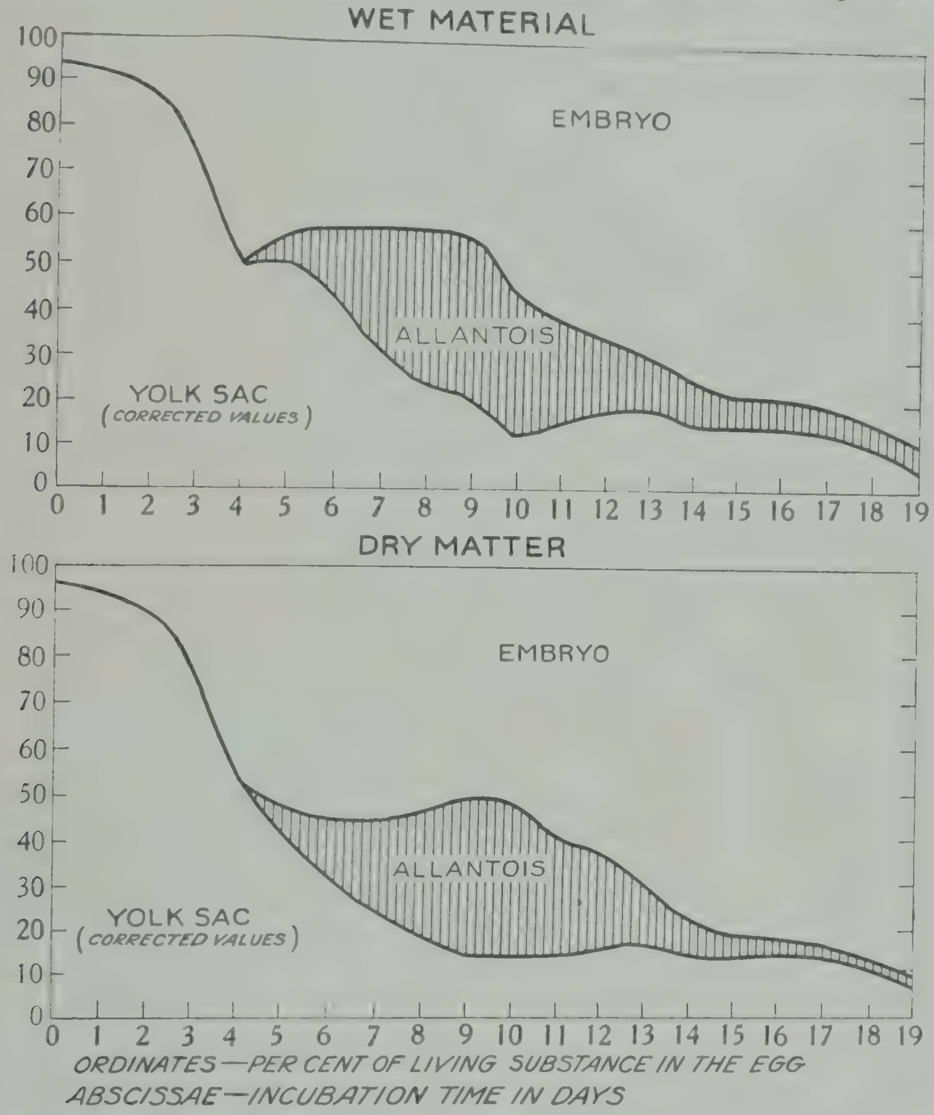


Fig. 25. Distribution of living matter in the developing hen's egg.

mbryo itself (whether considered as wet or dry weight) attain a bulk equal to nore than half the total living matter in the egg. This is a remarkable illustration f the importance of the scaffolding mechanisms required by highly lecithic, leidoic, terrestrial eggs.* For determinations of ash and total nitrogen in the nembranes we are indebted to Penquite. Fig. 26 shows from a different angle, hat of nitrogen storage, the importance of the membranes in the earlier stages.

Compare with this the closely analogous role of the pulp in avian feather development (F. R. illie[10]). And the few quantitative data that we have on the development of galls (see p. 106) ndicate that the growth of the gall is at first enormously greater than that of the nymph, while ater it almost ceases though that of the insect continues (B. W. Wells). Here, of course, the iology of the process is primarily protection, not nutrition.

When mgm. total N in the membranes are plotted against mgm. total N in t embryo on a double log. grid, it can be seen that not until the 15th day does t

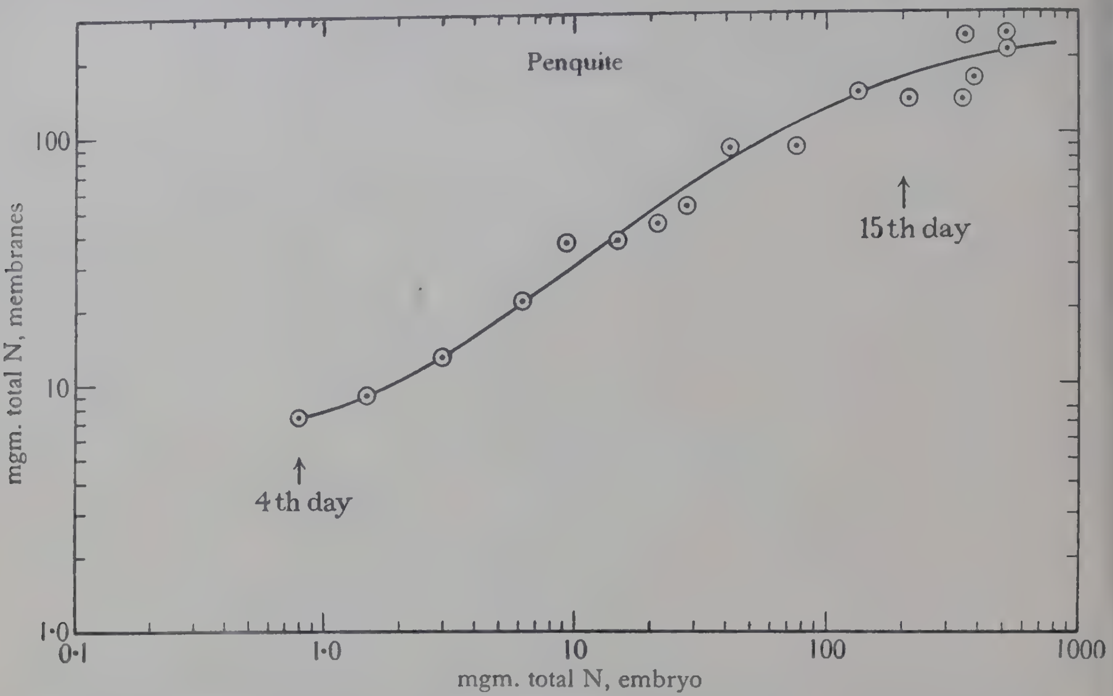


Fig. 26. Relation of nitrogen storage between chick embryo and its membranes.

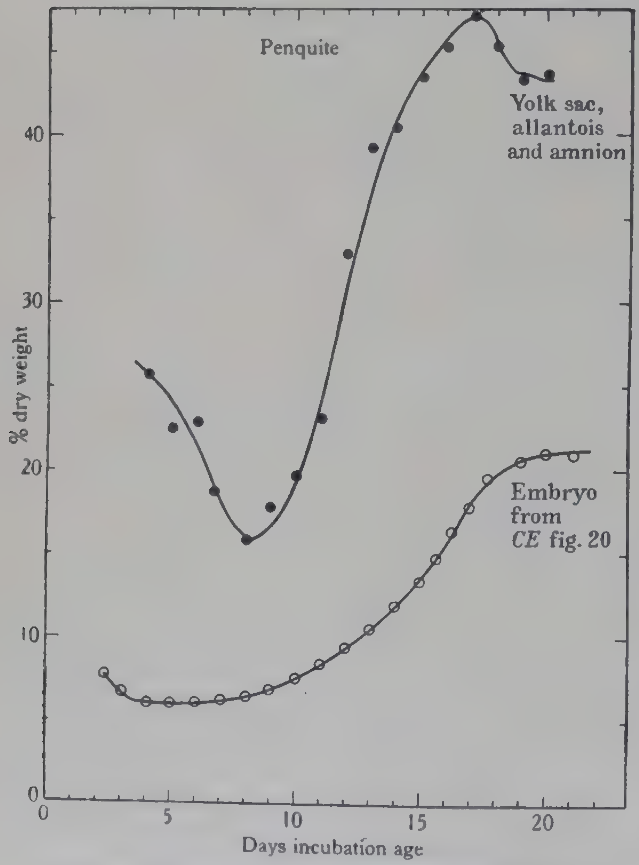


Fig. 27. Hydration and dehydration of the chick embryo's membranes.

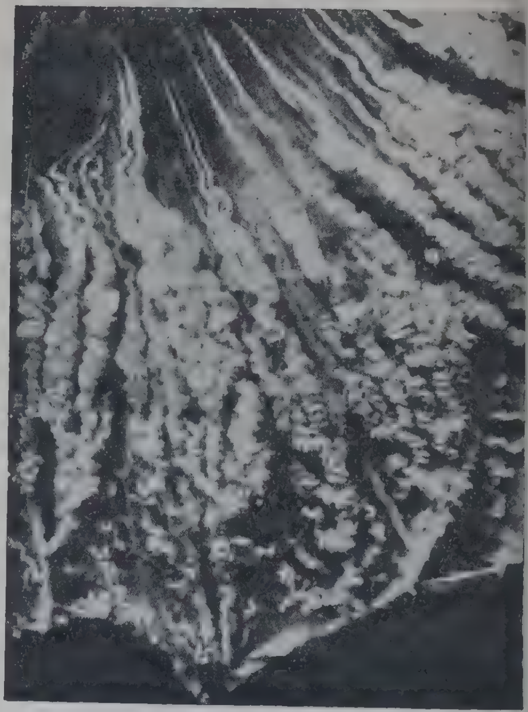


Fig. 28. Interior surface of chick yolk-sac showing villous blood-vessels.

embryo overhaul the membranes (embryo 200 mgm., membranes 160 mgm.). The curve then approaches the horizontal, for the embryo continues to grow hile the membranes do not. Earlier, at the 4th day, the embryo has ten times ss nitrogen than the membranes.

As a result of Penquite's work we know now the percentages of dry weight and h in the embryonic membranes. Fig. 27, calculated from his data, shows that like the embryo, which as is well known ows a progressively rising dry weight %, e yolk-sac's dry weight % passes through minimum on the 8th day, rising thereter to a point near hatching at which early half its weight is dry substance. his is no doubt connected with the resence of such large amounts of raw aterials from the yolk being absorbed by e yolk-sac and transmitted to the embryo, nd it is interesting that the maximum ydration of the yolk-sac occurs during the eriod of its maximum growth rate. As the olk-sac ceases to grow, and when the yolk as been fully enclosed, a mass of raditing lamellae or villi appear on its interior urface; these play an important part in the take of food, and are illustrated in Fig. 28, ken from Remotti's study of them[3].

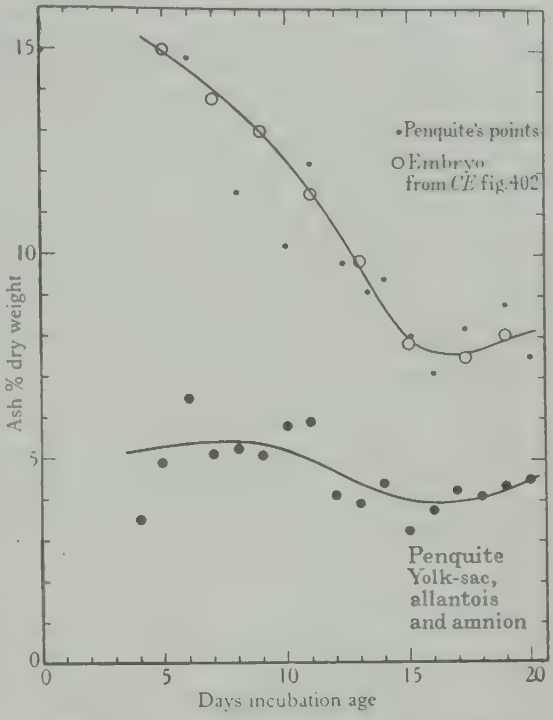


Fig. 29. Ash content of chick embryo and membranes.

During its development the chick embryo like all other embryonic organisms, cf. . 546) becomes progressively poorer in ash, related to dry weight. As Fig. 29 ows, however, the content of the membranes in inorganic substances remains ughly constant (Penquite).

A comparison of the absorptive work carried out by the yolk-sac and its etabolism was made by Needham[6]. Defining the Membrane Absorption Rate as

$$\frac{y_i+a_i+e_i+c_i}{y+a}\times 100,$$

nd the Membrane Transit Rate as

$$\frac{a_i+e_i+c_i}{y}\times 100,$$

vhere y_i is the daily increment (wet weight) of the yolk-sac,
a_i is the daily increment (wet weight) of the allantois,
e_i is the daily increment (wet weight) of the embryo,
c_i is the daily increment of material combusted and lost from the egg,
y is the wet weight of the yolk-sac,
a is the wet weight of the allantois,

t was possible to calculate the amount of material retained each day for the onstruction or maintenance of the yolk-sac and that passed on for the construction and maintenance of the embryo. At the beginning of development the yolk- absorbs more than three times its own weight of material each day, but by 9th day an equilibrium has been attained and after that time it absorbs abou own weight of material each day. The high Absorption Rate of early developm is almost exclusively concerned with the construction of the yolk-sac itself. T Transit Rate indicates that after the 9th day almost all the material absorbe transmitted to the embryo.

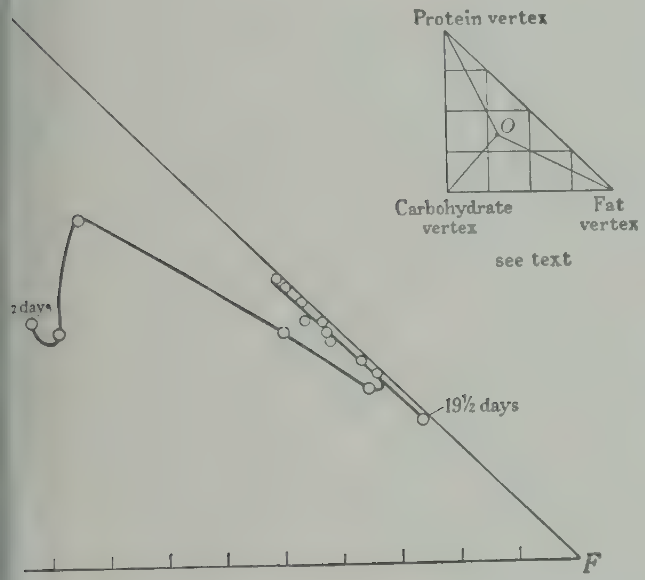
Respiratory quotients of all the membranes were measured in glucose medi each day during development[7,14]. As Fig. 316 demonstrates, the R.Q. of the yolk- falls from about 0·9 on the 2nd day to 0·6 on the 7th, after which it rema approximately constant a little below 0·6 till the 14th. After the 14th d accurate quotients are difficult to obtain, because the respiratory rate is very l and the bound CO_2, probably because of localised deposits of calcium carbona very high. Nevertheless, it is clear that there is a correlation between the grow period of the yolk-sac and its metabolism, for while it is rapidly growing a developing, its R.Q. is high, but when it ceases to do so and begins to pass on that it absorbs to the embryo, its R.Q. is very low. These low quotients were foun not to be due to the conversion of fat to carbohydrate or the desaturation of fat acids or the formation of keto-bodies. They probably indicate a catabolism of f with certain modifications.

In the same diagram data regarding the R.Q. of other parts of the egg syste will be found. The R.Q. of the allantois falls slowly from about 0·95 on the 5t day to about 0·82 on the 20th. At the time of its origin from the embryo, therefore its catabolism most closely resembles that of the embryo, and later progressivel departs from it. So long as the size of the embryo remains sufficiently small t allow of the manometric measurement of its R.Q. this is found to be unity, bu later on the R.Q. must fall, for at the time of hatching it is certainly in the neighbourhood of 0·7. This subject will be referred to again later (p. 592).

A good deal of work has been done on the histology of the yolk-sac. It contains cells of reticulo-endothelial type and cells which phagocytose (Coppini; Zanoni; Steinmüller) and has been studied in tissue culture (J. A. Thomas[3,4]). It may continue to live for some time after the death of the embryo (Remotti[4]). Its vascular cells have peculiar physiological properties (Nakano).

We come now to the enzymic equipment of yolk, white and yolk-sac in relation to the absorption of the raw materials. As we have already seen, the materials which enter the embryo are, after the 5th day, fat and protein in overwhelming preponderance. This may be illustrated in an interesting way by the method of I. Fisher. If a right-angled triangle is drawn, the three vertices representing protein, carbohydrate and fat, the composition of any given diet may be represented by a point. In the case of the point *O* in the small diagram of Fig. 30, representing a given percentage of fat and protein, the proportion of carbohydrate in the diet is represented by the area of the triangle *OPF*, and the larger the proportion of carbohydrate in the diet, the nearer *O* would approach *C*. Similarly, the proportion of protein in the diet is given by the area of the triangle *OCF* and that of fat by the area of the triangle *OCP*. Passing to the large diagram lculated from *CE*, Section 6·9), it will be seen that the diet of the chick embryo ly contains appreciable amounts of carbohydrate in the early stages of developnt. About the end of the 1st week the line of points approaches the *PF* line d there oscillates during the remainder of incubation in the manner shown in g. 30.

As stated in *CE*, pp. 1303 ff., proteases and lipases have long been known to cur in the yolk and white. Yolk-protease is believed to reach a maximum of tivity about the 10th day, i.e. at the time of maximal protein catabolism emotti[1] on hen's eggs; Calzoni[1] on duck's eggs), and yolk-lipase about the 16th y, i.e. the time of the second maximum of fat absorption. Amylase is also esent in yolk (Tateishi[2]), and white (Rotini).



ig. 30. Diet of the chick embryo (I. Fisher's method)—in gm. not calories.

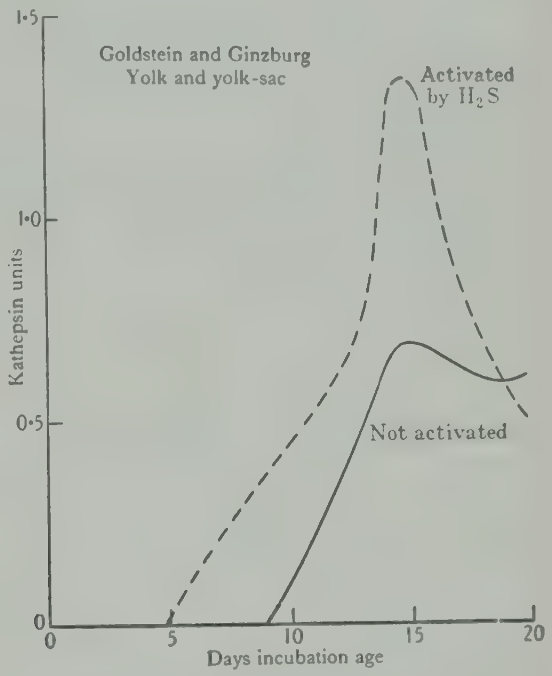


Fig. 31. Kathepsin activity of chick yolk and yolk-sac.

The kathepsin activity of the yolk and yolk-sac together is shown in Fig. 31. his work (Goldstein[2]; Goldstein & Ginzburg; Goldstein & Millgram) shows at the activity of the enzyme increases up to the 15th day and then remains nstant, though if the activity realisable by activation with H_2S is considered, ere is a peak of activity at the 15th day. This picture so much resembles that f the growth of the yolk-sac itself that the latter is probably the largest factor n it. All investigators are agreed that during the first half of development the ctivity of the proteolytic enzymes in the yolk-sac is much greater than that of hose in the embryo. Thus Borger & Peters found that the proteinase of the olk-sac (optimum *p*H 4·0) on the 8th day was twelve times as active as that of he embryo (in the allantois practically absent), and Krebs found a P.Q.* of 13·2

$$\text{P.Q.} = \frac{\text{c.mm. amino-N}}{V \dfrac{a}{(a+b)\,x} \times \text{hr.}}$$, where V is the vol. of the active extract, a the vol. of tissue used, b the ol. of glycerol used, and x a factor depending on the percentage water in the tissue.

in the yolk-sac of 8-day embryos as against 1·24 in the embryos themselv Mystkowski[2] found a P.Q. of about 2·5 (unactivated) and 6·5 (activated by cyste both at early stages (4 days of incubation) and at late ones (18 days) for the yo sac, as against 0·25, unactivated, and 0·35, activated, for the embryo itself. T same is true of dipeptidase. Found by Borger & Peters in the yolk-sac, and a slight extent in the allantois, it was estimated by Donegan as about three tim more active on the 5th day than that in the embryo. There is also amin polypeptidase in the yolk-sac (Borger & Peters). We may conclude that the hea burden of digestive work borne by the yolk-sac in the earlier stages involves powerful enzymic equipment.

The fact that some of the kathepsin in the yolk-sac can be activated b sulphydryl shows that a certain reserve normally exists. Goldstein & Ginzbur found that the ratio between the unactivated and activated values was the same in the chick's yolk-sac as in the placentas of several mammals (guinea-pig, rabbit and rat). Goldstein[2] has brought forward evidence to show that yolk-sac kathepsin acts more strongly on proteins from the egg than on any others save gelatin.

There are enzymes concerned with nitrogenous substances in the yolk-sac, however, which cannot be activated by sulphydryl, e.g. arginase (Needham, Brachet & Brown). The amount of arginase, in any case, in the yolk-sac is much less than that in the embryo (at the 5th day, e.g. $Q^{38^\circ}_{\mathrm{H}}$ 0·32 for the embryo, 0·025 for the yolk-sac).*

The lipases in the egg, particularly esterase (methylbutyrase) and lipase (tributyrinase), have been the subject of an interesting study by Ammon & Schütte. The unincubated yolk has more of these enzymes than the white; yolk esterase 46 W,† yolk lipase 88 W; white esterase 5 W, white lipase 15 W. No change occurs in the enzymes of the yolk or white on incubating eggs, whether fertilised or not, but the activity of both enzymes rises regularly in the developing embryo, as shown in Fig. 32. But in the yolk-sac the activity is particularly marked; it is many times higher than the embryo in the early stages, and thirty times that of the yolk, even at the earliest stages (see Fig. 32). In the allantois it is low and remains relatively constant at esterase 50 W and lipase 300 W. That there is a synthesis of the enzymes as a whole appears from the number of units

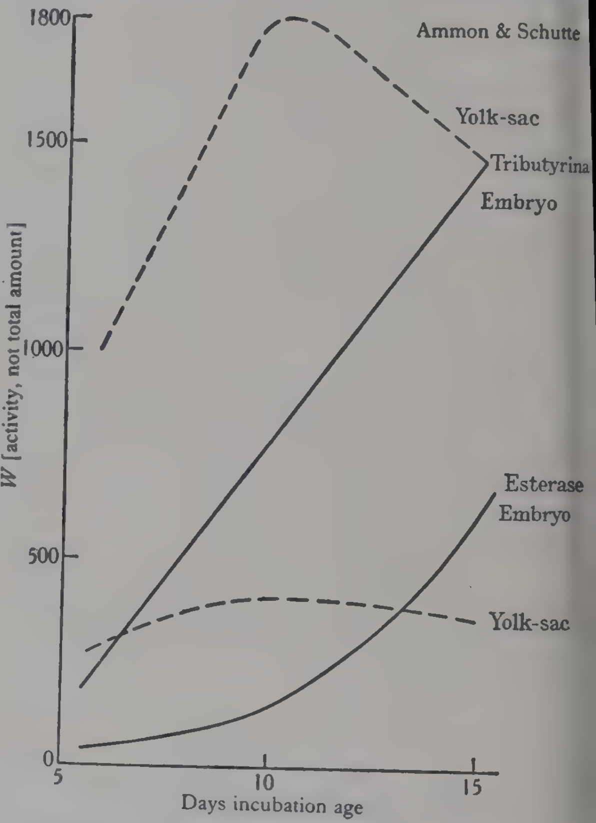


Fig. 32. Lipase activity of chick embryo and yolk-sac.

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* $Q^{38^\circ}_{\mathrm{H}} = \frac{\text{c.mm. urea } CO_2}{\text{mgm. dry wt.} \times \text{hr.}}$ (manometrically).

† W = units/c.c. extract.

ntained in the entire egg system, which rises from 132 to 1125 in the case of terase and from 264 to 3102 in the case of lipase.

Choline esterase, on the other hand, rises in activity in the yolk itself (from W to 294 W on the 15th day) as well as in the embryo (392 W to 1120 W). the yolk-sac it remains constant at 170 W and in the allantois at 100 W (cf. the ɔrk of Nachmansohn referred to on p. 643). Physostigmine inhibits it.

Enzymes of the egg-white have not been much studied recently. Apart from e older observations mentioned in *CE*, p. 1304, van Manen & Rimington have cognised two erepsins (*p*H optima at 5·5 and 7·5) and deny that egg-white is ıtolysable, while Balls & Swenson think that the thick egg-white can autolyse ıd contains a trypsin-like proteinase. The last-named authors have studied a yptic inhibitor present in the thin egg-white, which they believe to be a sulphur-ɔntaining polypeptide, the action of which can be reversed by adding entero-nase.

This is the place to mention the very remarkable substance lysozyme, a acteriolytic protein of low molecular weight with the properties of an enzyme, hich occurs richly in egg-white, and has lately been crystallised (Abraham & obinson). Lysis manifest to the naked eye is performed by egg-white diluted in 1 million. Details concerning it will be found in the excellent review of leming, but what part any of these substances play in embryonic metabolism still quite obscure.

Utilisation of the raw materials proceeds most efficiently at the optimum mperature of development (Penionskevitch; Romanov, Smith & Sullivan). At ıe end of the incubation period the yolk-sac does not degenerate; the vessels mply constrict and the tissue dies "in complete possession of its physiological ritability and anatomical integrity" (*CE*, p. 725). The process of this atrophy d means of preventing it have been studied in detail by Remotti[3]. The removal the "spare yolk" (*CE*, p. 939) with which the embryo hatches, sometimes lled "deutectomy", by no means leads to its death. Barott, Byerly & Pringle ıve shown that in that case the chick simply lives on the reserves of its own ɔdy, and loses more weight than normal chicks at all temperatures observed. s R.Q. also is unaffected. But whereas the heat production of normal chicks is ɔnstant for a given temperature, in chicks without spare yolks it continuously lls off. Of course if no food and water are received from without, after 3 days ıe chick enters a starvation phase, and its body temperature begins to drop Card).

Of the amniotic membrane little is known. Remotti[5] assesses the endoamniotic ressure at 5·5 cm. Hg; it does not alter during development and is partly due ɔ a tonus of the muscle fibres in the amnion. Up to the 17th day there is a rogressive increase in its mechanical resistance and a progressive decrease in s speed of autolysis, but after that time the process is reversed and there is a apid change of both properties in the opposite direction.

1·82. Yolk utilisation in the lower vertebrates and invertebrates

We have very little information about the process of yolk utilisation in animals ther than birds. Except for what has so far been said about the selachians ɔ. 40), there is only a note by v. d. Ghinst[3] on the trout, reporting the presence f amylase in the syncitial vitelline cells at the blastula stage. For the amphibia, apart from the rather enigmatic cytological work of Kedrovsky and the Ko packis, there seem to be only the observations of Dorris, who combined delic micro-estimations of enzyme activity with histological sections of the develop gut in the axolotl. Amylase is the first enzyme to appear, at Harrison stage when the gut is still histologically undifferentiated. The proteolytic enzym pepsin and trypsin, appear at stage 43, just as the differentiation of the stoma glands and the acini of the dorsal pancreatic rudiment are coming to completi Such enzymes will account for the digestion of what remains of the yolk, but the processes going on in every cell of the embryo at much earlier periods know literally nothing.

A quantity of miscellaneous data about the utilisation of yolk in the silkwor egg has been collected in a review by Tirelli[5].

Yolk utilisation probably accounts for a very peculiar phenomenon the meanir of which is at present obscure, namely, the rhythmic contractions of the fi blastoderm from an early stage in development. These were first extensive studied by Ransom in 1854. Formerly (*CE*, p. 897) this was mentioned und the heading of water absorption, it being thought to have some connection wi the need of non-cleidoic embryos for water, but we now know that the contractio of micro-fibrils plays a quite fundamental morphogenetic role in insect development (see p. 463) and may even be not without importance in that of amphibi (see p. 146). However, since only the highly lecithic types of egg among the fishe show it, it is probably a stirring mechanism.

The most thorough study of blastoderm contraction in a teleost is that o Yamamoto[1] on *Oryzias latipes*. Rhythmic waves of contraction are seen first whe the blastoderm has enclosed nearly one-third of the egg, and the embryoni shield has just become visible. The blastoderm does not maintain a fixed hemispherical shape, but fluctuates continually. Waves of origin dextral to th embryonic axis alternate with waves of origin sinistral to it, though sometimes both may occur at the same moment and progress till they meet. Cell boundaries in the blastoderm, straight when at rest, become wavy on contraction. On studying the effects of temperature, Yamamoto obtained for the frequency of contraction, temperature characteristics (see *CE*, p. 524) none of which were the same as those typical either of rhythmic processes or of oxidation reactions, but which more resembled the figures for rate of embryonic development. When the temperature characteristics for the velocity of the wave and for the pause between waves were obtained, it was found that as temperature rises the time required for each wave to travel over the blastoderm decreases, and the time taken by each pause also decreases; in such a way that the frequency is the intermediate between them. The meaning of the temperature characteristics could, as usual, not be clearly ascertained. The effect of most ions in concentrations above the normal was to accelerate the rate of contraction and finally to stop it; this result is reversible if the embryos are returned to normal conditions before too long a time of exposure. Osmotic pressure, on the other hand, had no effect. Intermediate *p*H levels produce no effect, but at *p*H 3·0 the movements are accelerated, then stopped; at *p*H 11·0 the movements are slowed, then accelerated, then stopped. CO_2 first accelerates, then slows and stops. In anaerobic conditions the movements can continue for about $1\frac{1}{2}$ hr. but eventually come to an end. Even after 20 hr. anaerobiosis they may be resumed and development continue normally. Some *r*H indicators are reduced before the movement stops; others afterwards, thus fixing the point of cessation of movement at *r*H 6–9. As in muscle the energy required for a certain amount of contraction may be produced anaerobically, but the process cannot continue indefinitely without oxygen.

These data, though interesting, do not answer the one essential question, n normal development go on in the absence of the contractions? While it may that they are mainly connected with the absorption of food material from the k, we cannot as yet deny them a morphogenetic significance.

Apparently many other fishes exhibit the phenomena. They have been described the goldfish *Carassius auratus*, where the protoplasmic peripheral layer contracts fore the formation of the blastoderm, and the trout, by Yamamoto[2] (with view of the literature); in the stickleback *Gasterosteus aculeatus*, where even blastomeres contract (Wintrebert & K. C. Yung; Painlevé, Wintrebert & C. Yung); in the pike *Esox lucius* (Kasansky); and newly in the eggs of azilian fishes such as *Trachycorystes* and *Hoplias* (v. Ihering). They have been ned by W. Kuhl. They probably account for the rhythmic changes in electric pedance which trout eggs show (Hubbard & Rothschild).

Among the embryos of invertebrates all kinds of movements occur, ranging from amoeboid motion of the eggs of *Hydra* to the slow pulsations in the eggs of olluscs (*Limnaea*; Comandon & de Fonbrune[2]) and of worms (*Nereis*; Hoadley[6]). e are still far from any understanding of their meaning, but it will be remem-red that protoplasmic contractility plays a large part in echinoderm fertilisation, in the starfish "fertilisation cones" studied by Fol; Chambers[1]; Sugiyama[2] and hers.

1·83. Avian "milk" and mammalian colostrum

Since the time of John Hunter's essay "On a Secretion in the Crop of Breeding geons" in 1786, it has been known that in the development of nidicolous birds, ch as the pigeon, which do not hatch from their eggs in a fully developed ate, the embryos rely not only on the yolk and white but also on a special cretion which they receive from their parents after hatching. This so-called igeon milk" is a white slimy substance secreted by a special area in the gestive tract of both parents, the crop gland, and regurgitated to the squabs ams & Meyer). It thus represents a "histotrophe" for the embryo prepared a site other than the genital system (see pp. 42, 81).*

Analyses of pigeon milk by modern methods have frequently been made. l investigators (Dąbrowska; Carr & James; Reed, Mendel, Vickery & Carlisle; alzetto & li Volsi) are agreed that it has the following approximate composition:

	%
Water	65–81
Protein	13·3–18·8
Fat	6·9–12·7
Ash	1·5

is therefore a very much more efficient nutriment than mammalian milk, r its composition resembles that of rabbit or cat milk, yet the pigeon squab ubles its hatching weight in only 2 days, whereas the rabbit doubles its birth eight in 6 and the cat in 9. Pigeon milk contains a large amount of desquamated

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Can it be that the gelatinous cement of the nests of certain swifts (Cypselidae), *Collocalia* spp., ich has rendered them a famous Chinese delicacy, recommended by traditional dieticians for its tritional properties when made into soup (*yen wo t'ang*), is really a crop-gland secretion? If , the gland would thus subserve another phase of reproductive activities.

cell material, which incorporates much of the fat. The hypertrophy of the cr to form the crop gland is remarkable. According to Carr & James the pare birds can be fed on maize while the squabs are being fed from the crop glan which argues a synthesis of essential amino-acids in the organ. The pigeon m contains no carbohydrate (Dąbrowska), but has active amylase and inverta (Dulzetto & li Volsi). It also contains adequate supplies of vitamins A, B_1 ar B_2 (Reed *et al.*). Its ash (Białaszewicz & Lewis; W. L. Davies) differs great from the ash of mammalian milk, having much more sodium and much le calcium. Dulzetto & li Volsi found the low iodine value of 40 for the fatty aci in it, and recognised the larger part of the protein as a phosphoprotein. Th presence of serum vitellin in the blood of the *male* pigeon at such times shoul therefore be worth looking for. The squab gets up to 10 gm. of the milk a day

A good deal of work has been done on the endocrine control of the crop glan Like lactation proper, the crop-gland secretion is stimulated by prolactin, th mammotrophic hormone of the anterior pituitary (Riddle & Braucher; Riddle Bates & Dykshorn; Bates, Riddle & Lahr), which is also responsible for inducin broodiness in birds (Riddle, Bates & Lahr) and which begins its action on the crop by stimulating mitoses in a certain area (Lahr & Riddle), though this actio could not be reproduced *in vitro* (Salle & Schechmeister). Prolactin is a protein, very heat-stable and of low molecular weight (Riddle; Bates & Riddle; Bates, Riddle & Lahr). The amounts of it required are exceedingly low (Lyons & Page; Lyons). Hypophysectomy greatly reduces (Schooley, Riddle & Bates) or abolishes (Gomez & Turner) the response of the presumptive crop gland, but adrenalectomy and thyroidectomy have no effect on it. Castration of the male eventually stops the reaction (Patel; Kaufman & Dąbrowska), but only after some time and as the result of the changes in the testis, since the crop gland will still respond to prolactin (Riddle & Dykshorn). Lastly, like lactation proper, the crop-gland secretion is inhibited by oestradiol monobenzoate (Folley & White).

The significance of "pigeon milk" cannot be understood save in the light of the evolution of the sauropsida, well discussed by Portmann[3]. The nidifugous habit, where the embryo is hatched as nearly alike to the adult bird as possible after a relatively long incubation period, is undoubtedly primitive. Portmann divides the birds into the following groups:

Table 12. "*Larval compression*" *in Birds*

	Time of learning flight	Condition of feathers	Visual condition at hatching	Nutritional dependence on parents	Example
1. Nidifugous	Quick	Feathers	Can see	None	Fowls, etc.
2. Nidifugous	Quick	Down	Can see	None	Geese, etc.
3. Nidifugous	Slow	Down	Can see	Present	Seagulls, etc.
4. Nidifugous	Slow	Down	Can see	Marked	Birds of prey
5. Nidicolous	Very slow	Down	Blind	Marked	Penguins, petrels
6. Nidicolous	Very slow	Reduced down	Blind	"Milk"	Pigeons, etc.
7. Nidicolous	Very slow	Very reduced down	Blind	Chirp reflex, regurgitation, etc.	Singing-birds, sparrows, etc.

He has himself studied the relative growth of organ weights in nidicolous as compared with nidifugous birds (Portmann[5]), from which it is clear that

e are considerable differences. It should be noted that in mammals there is a
allel series (cf. Table 13). The epithelio-chorial placenta, with its long gestation
e and fully developed foetus at birth, contrasts with the haemo-chorial type
p. 81) with short gestation time (apart from man, a very exceptional case)
undeveloped foetal condition at birth. There is also a parallel in that some
mmals give substances of importance to the young animal through the
ostrum after birth while others do not, but the parallel cannot be carried
ther because colostrum is associated rather with the epithelio-chorial placenta
account of what, owing to its many cell-layers, it has failed to let through,
n with the haemo-chorial placenta, transmission through which is very efficient
E, p. 1497). A concrete instance is that of vitamin A, which, as Dann[2,3] has
own, is contained in high concentration in the colostrum of ungulates such as the
x (epithelio-chorial placenta) where 1 day's feed of colostrum is equivalent to
days of milk, but only insignificantly in human colostrum (haemo-chorial
acenta). Even in human milk, however, there may be substances supplementing
infants' capabilities, for it is said that at first salivary amylase is aided by
ylase in the milk (Schlack & Scharfnagel). All kinds of enzymes—catalase,
ylase, invertase, monobutyrinase, tributyrinase, etc.—(Katsu) are present
human colostrum (analyses by Widdows, Lowenfeld, Bond, Shiskin &
ylor).

In sum, therefore, among both birds and mammals "larval forms" are to be
und, though never so morphologically deviating from the adult form as in the
ver vertebrates and invertebrates.

1·84. Maternal diet and embryonic constitution in mammals

nder the general heading of nutrition the question may well be asked whether
l to what extent the nutritional conditions prevailing during intra-uterine life
mammals affect the growth and development of the foetus. This raises a
blem analogous to that discussed in connection with the chick embryo in
ction 1·3. Avoiding the rather large and unsatisfactory semi-medical literature
ntion will only be made here of a few orienting papers (e.g. for the mouse,
duhr; for the rat, Wan & Wu; for the rabbit, Rosahn & Greene; Rosahn,
eene & Hu; Kopeć; Kopeć & Latyszewski; for the pig, Mitchell, Carroll,
milton & Hunt; for cattle, Eckles; Haigh, Moulton & Trowbridge).

In general it may be said that mammalian development is an all-or-none
enomenon. It may be interrupted by insufficient calorie intake or lack of one
other of the essential dietary materials in the food of the maternal organism;
this case foetal resorption (see p. 65) or abortion will ensue. For example,
is will happen in the rat if there is less than 5 % protein in the diet (Guilbert
Goss). But if the process can be carried to its normal termination at all, the
mposition and viability of the embryos will be approximately normal. In order
study the effects of extreme under-nutrition during pregnancy, Jonen subjected
egnant rabbits to absolute fasting for various periods, with the result that at
rth the weights of the foetuses were much more abnormal than their chemical

composition. Diminutions of weight of 33 % were not uncommon. But as following figures show:—

At the 24th day:	Foetus			Maternal organis
	Wet wt. gm.	Dry subst. %	Fat % dry wt.	fat % dry wt.
Normal	11·21	12·48	23·1	48·06
Starvation for 10 days during pregnancy	7·43	12·82	19·0	33·0

there may be a great diminution in total weight while the relative amount dry substance in the foetus remains unchanged and the fat-content is or diminished to a minor extent. The fat reserves of the maternal organism will of course much depleted. On the other hand, if the starvation period ceases la in pregnancy, it may be too late for the foetus to be able to take advantage of t nutrient materials now newly circulating in the maternal blood.

Conditions so extreme are never met with in man, though a seasonal variatio in birth weight has been reported by Toverud; Abels, and others; infants bor during late summer and early autumn being significantly heavier than those bor during other months.* Such slight changes will always be difficult to establis and hard to interpret. Sontag, Pyle & Cape could not observe any differences i birth weights according to the dietary level of the mother, and German authors i general regard the birth weight as not being influenced by the maternal diet, eve during war famines. Nevertheless, Gerschenson, basing his view on evidenc collected in South Russia, where about 1917 the war famines were much worse tha in Germany, has shown that very severe hunger conditions do reduce the birt weight in man. In general, mammalian development may be regarded as a all-or-none phenomenon provided the limits of variation of the maternal die are small; if they exceed a certain limit, then the regulating mechanisms break down and such embryos as are born will be markedly abnormal.†

Very few experiments to raise artificially some foetal constituent have been done. From the work of Dann[1], however, we know that a great excess of dietary vitamin A, though it may enrich the maternal liver of the rat, cannot increase the store in the foetus. Only after birth can it pass through to the young animal in abnormally large quantity via the milk.‡

For human pregnancy there have been a number of studies dealing with the utilisation of dietary constituents by the foetus; it will be sufficient to refer here to those of the Michigan school (Macy & Hunscher on nitrogen and minerals; Macy, Hunscher, Nims & McCosh on calcium; Hunscher, Donelson, Nims, Kenyon & Macy on nitrogen storage; Hummel, Hunscher, Bates, Bonner & Macy on metabolic balance; and Johnston, Hunscher, Hummel, Bates, Bonner & Macy on basal metabolic rate), through which the literature can be reached, as also through the descriptive bibliography of L. Zuntz[4].

* Cf. the mysterious seasonal variation in twin births established by Cole & Rodolfo in cattle, where there is a maximum in August and a minimum in March.

† It is interesting that according to Enzmann, Saphir & Pincus the marked delay in pregnancy caused by lactation does no harm to the blastocysts whose implantation into the uterine wall is delayed. If the delay lasts longer than a certain time, however, the blastocysts degenerate and never become implanted.

‡ The importance of each vitamin for the maintenance of foetal development has been dealt with in a useful review by K. E. Mason[2].

astly, it has been conclusively shown that in multiparous animals there is a nite relation between litter size and birth weight (Wishart & Hammond; mmond[2]; Watt; Crozier & Enzmann; Enzmann & Crozier). There seem to be tain strict limitations on the amount of material which the maternal organism given species can devote to embryonic growth and therefore the larger the nber of embryos in the litter the smaller each one of them is.

. The mammalian placenta

The tendency to retain the egg and embryo for protection during its developnt within the maternal body, and to furnish it with extra nourishment, whether water, salts or organic substance, not provided in the yolk, comes to its climax the mammalian placenta. In morphological complexity and physiological erest this organ is unique, and it can be no matter for surprise that it has given

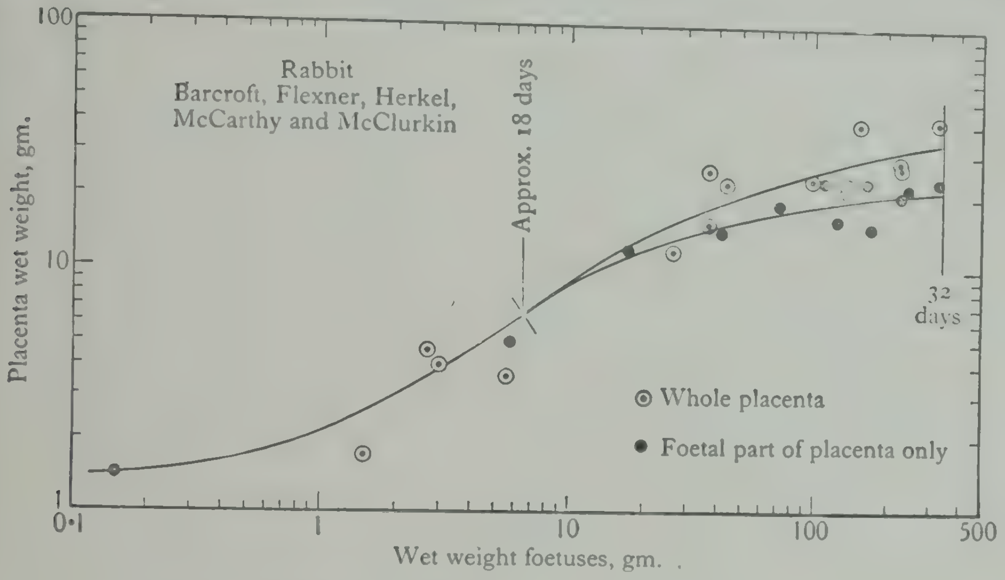

Fig. 33. Relation between rabbit embryo and placenta.

th to a vast literature, partly, of course, with a medical bearing, but also largely product of a genuine biological outlook. Since the placenta is of mainly tritional significance and has relatively little to do with morphogenesis, we nnot allow it much space in the present book, but by referring to reviews and those papers which themselves contain summaries of previous work, access to literature may be facilitated for those specially interested in placental problems.

As we have already seen (p. 67), the yolk-sac and other membranes in the gs of other amniotes, such as the chick, at first outstrip in their growth the nbryo itself. The same process takes place in mammals. Fig. 33, constructed om the data of Barcroft, Flexner, Herkel, McCarthy & McClurkin, shows the lation between the embryo and the foetal and maternal parts of the placenta in rabbit; it is directly comparable with Fig. 26, for it is plotted on a double garithmic scale. It will be seen that during the first half of development the centa weighs much more than the foetus, but that about the 18th day, at a ight of 6½ gm. each, the foetus overhauls the placenta and the curve then ttens out as the embryo continues to grow much faster than its scaffolding.

A similar set of data has been published for the rabbit by Hammond[3]. Hammon figures for the cow are shown in Fig. 34. The relationships are the same—equ in weight between foetus and placenta is reached at a weight of about 300 after which the curve flattens, but equality in weight between the foetus and foetal liquids (amniotic and allantoic) is not reached until a much later stag development, at a weight of some 5 kg. The significance of the deviating point for the placenta is not clear. Similar graphs could be made for all mamm see e.g. the data on the merino sheep (Cloete; Malan & Curson).

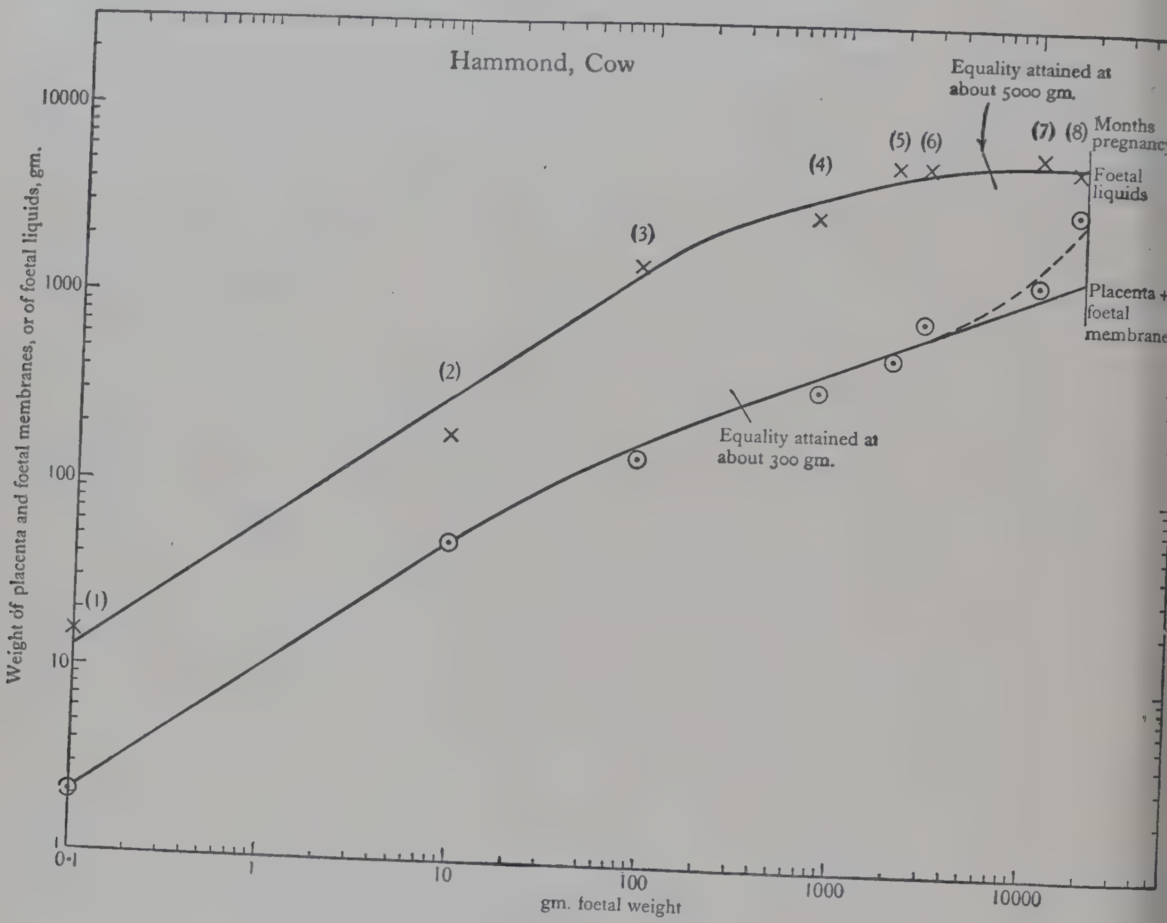

Fig. 34. Relation between cow embryo and placenta.

There is a marked positive correlation between foetal weight and placenta weight at term in the rabbit (Rosahn & Greene; Hammond[3]), in man (de Aberle, Morse, Thompson & Pitney) and probably most mammals.

Although the placenta in many animals gives the impression of being a new organ, it is after all no more than the tissue formed at the points where the two blood circulations of embryo and maternal organism come into contact. Since its significance is almost wholly nutritional, it is logical that the most satisfactory classification of placentas should be a physiological one, and this admirable work was accomplished by Grosser[1,3] in his distinction between the number of membranes or cell-layers which intervene between the two circulations. The following table (simplified from *CE*, Table 223) illustrates his scheme, somewhat modified by later writers.

Table 13. *Placenta types*

Type of placenta	Maternal blood	Maternal endothelium	Maternal mesodermal connective tissue	Maternal epithelium	Foetal epithelium	Foetal mesodermal connective tissue	Foetal endothelium	Foetal blood	Examples
		Maternal uterine mucous membrane			Foetal chorion				
Epithelio-chorial*	+	+	+	+	+	+	+	+	Horse, pig, donkey
Syndesmo-chorial	+	+	+	−	+	+	+	+	Cow, sheep, goat
Endothelio-chorial	+	+	−	−	+	+	+	+	Dog, cat
Haemo-chorial†	+	−	−	−	+	+	+	+	Primates, some rodents
Haemo-endothelial	+	−	−	−	−	−	+	+	Some rodents

ecent elaborate description by Brambel.

ecent elaborate description by Spanner and Kearns; tissue culture studies by Sengupta and gayama.

Ve have to deal, therefore, with a progressive breaking down of the barriers ween the foetal and the maternal circulations. It may be considered as ablished that the condition of the barrier, whether substantial, as in the thelio-chorial placentas, or very thin, as in the haemo-chorial and haemo-lothelial ones, may be correlated with (1) the transmissibility of substances of ous molecular weights from one circulation to another (*CE*, p. 1505), (2) the ence or absence of a large allantois as the receptacle for foetal excretions , p. 1495), (3) the relative significance of the uterine "milk"* (*CE*, p. 1492), the relative importance of the colostrum as the vehicle for substances of ortance which may not have been supplied sufficiently readily by the placental n (*CE*, p. 1497). In this connection it should be remembered that, as Mossman nts out, the placental type may change during the development of the embryo, inning with the looser connection and going on to a more intimate one. An eme example is the case of the rabbit, where at first the placenta is haemo-rial in type, but later becomes "haemo-mesothelial" and later still haemo-othelial. We have already had occasion to note in the work of Rodolfo the nificance which these changes may have in placental function (p. 31).

The question of the evolution of the placenta in mammals is a much disputed e. Benazzi, and Mossman, in his splendid monograph on the comparative

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his secretion from the uterine glands, the "histotrophe", is copious in those animals with helio-chorial placentas and scarce in those with haemo-chorial ones. Brambel and Hamlett[2] well shown how it accounts for the "hippomanes"; packets of uterine secretion surrounded rophoblast and invaginated into the allantoic cavity. There was a good deal of confusion ut these in antiquity as a glance at Aristotle shows (*Hist. Anim.* 572 a 21, 577 a 10, 605 a 2), like the uterine milk itself they were first properly described by Walter Needham in 1667. ir function, if any, is still obscure.

morphogenesis of the foetal membranes, regard the epithelio-chorial type as most primitive, and this is in accordance with what has already been said in t book regarding the placentas of dogfishes and reptiles (pp. 43, 61). Portman on the other hand, considers the haemo-chorial type to be the most primiti his arguments are criticised by de Lange.

Adopting here the former view, we can see that there were several directi in which the primitive epithelio-chorial placenta could evolve (see Fig. 35).

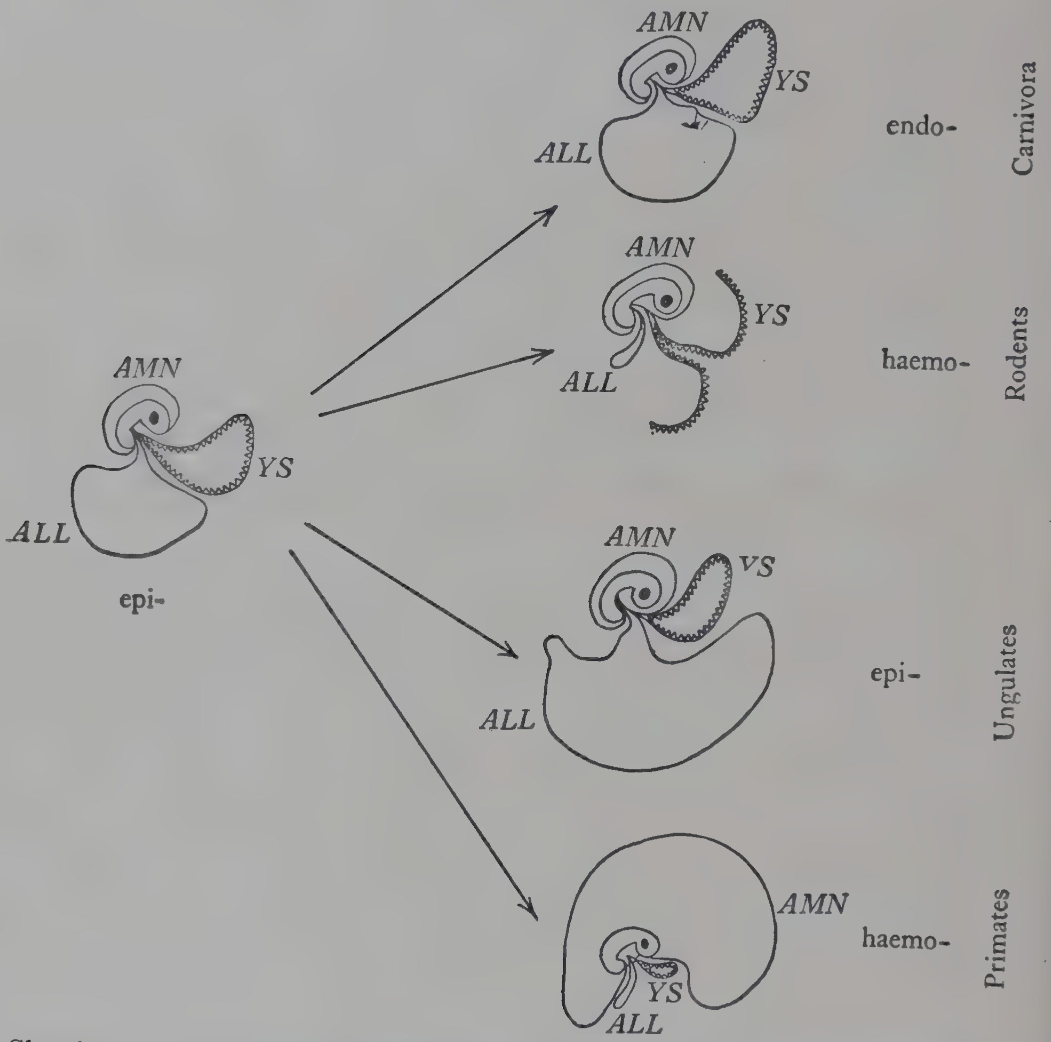

Fig. 35. Sketch of the origin of the several types of placenta. *ALL*, allantois; *AMN*, amnion *YS*, yolk-sac; after each hyphen read "-chorial".

probably had a large yolk-sac and large allantois, with chorio-allantoic attachmen to the uterine wall. Retention of these primitive characters with disappearance o maternal epithelium and connective tissue led to the modern carnivora. But now the allantois could be greatly reduced in size or, on the other hand, greatly enlarged. In association with the former tendency there went an opening up of the yolk-sa so that by the disappearance of its wall most distal to the embryo, the inner endodermal layers (with their ancient yolk-absorbing villi, cf. Fig. 28) could be apposed to the maternal uterine wall.* This process led to the haemo-chorial placentas of the insectivora and rodents. On the other hand, an excessive enlarge-

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* This is the famous "inversion of the germ-layers".

ıt of the allantois meant contact with the maternal tissues over a greater area
hence the avoidance of the necessity for a very close juxtaposition of the
ıds. This led to the modern epithelio-chorial and syndesmo-chorial placentas
he ungulates and all their related groups. But there was also a fourth possi-
y, namely to reduce drastically the size of both yolk-sac and allantois and to
blish over the chorio-amniotic area a close haemo-chorial connection. This
rse led to the primates and man (see Fig. 36).

he contact area between maternal and foetal circulations in man is very great;
istoffersen has recently assessed it at between 10 and 13 sq. metres, considerably
er than was formerly thought. The volume of foetal blood increases in
portion to the combined weight of foetus and placenta (Elliott, Hall & Huggett)
the relative amounts of it in foetus and placenta have been studied by

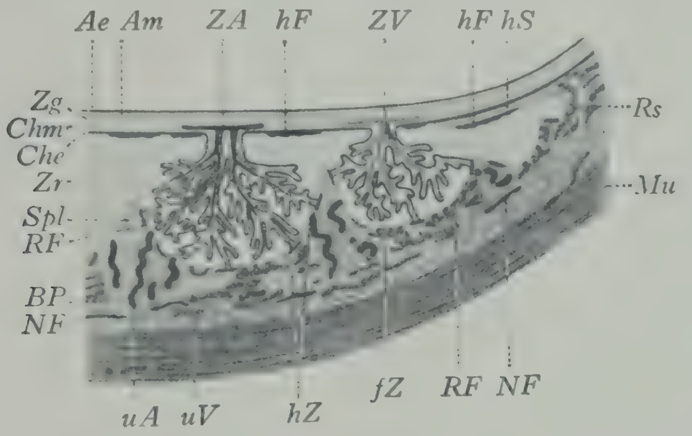

Fig. 36. Structure of the human placenta (from Fischel).

amniotic epithelium.
, amniotic mesoderm.
basal plate.
chorionic epithelium.
, chorionic mesoderm.
ree villus.
hypochorial fibrinoid striae.
hypochorial rim of uterine tissue.
anchored villi.
uterine muscle.
Nitabuch's fibrinoid striae.

RF, Rohr's fibrinoid striae.
Rs, peripheral blood sinus.
Spl, septum placentae.
uA, uterine arterioles.
uV, uterine venules.
Zg, loose mesodermal connective tissue between amnion and chorion.
ZA, foetal arteriole.
ZV, foetal venule.
Zr, intervillous blood sinus.

croft & Kennedy on the sheep. At 100 days about half the total blood volume
ı the placenta, at 120 days about a third and at 140 days about a fifth. This is
to the increasing total blood volume and foetal size; there is no diminution
the blood in the placenta. Some of the blood in the placenta at the end is
ed for the foetus (Barcroft & Gotseff). Grosser[2] has drawn attention to the
gnation of the maternal blood in a villous haemo-chorial placenta such as that
man, and to the contractions which squeeze the blood out of it like a sponge.
t as these may occur only once an hour, there is plenty of time for the action
placental enzymes on substances coming to the placenta from the maternal
od. In labyrinthine epithelio-chorial placentas no stagnation of this sort
urs. The mean volume of blood in the uterine vessels rises greatly during
elopment (from 2 to 32 c.c. in rabbits) parallel with the weight of the placenta,
t the embryo (Barcroft & Rothschild; Barcroft, Herkel & Hill). During

pregnancy the spleen contracts, presumably to supply blood to the ut (Barcroft & Stevens); this contraction is abolished by denervation (Barcroft

Contrary to the general belief that the chorio-allantoic and chorio-amni regions are the only important seats of exchange in mammals, Everett found vital dyes accumulate in the highly inverted yolk-sac placenta of the rat fr the maternal circulation. He made the interesting incidental observation on the 12th day of development toluidin blue is instantly reduced on enter the yolk-sac placenta.

Until recent times practically nothing was known of the experimental morph logy of the placenta. But starting from the pioneer experiments in which L. Lo in 1907 was able to produce the maternal part of the placenta in the guinea- by applying mechanical stimuli to the uterus in the presence of a favoura endocrine situation, the work of a number of investigators (reviewed by Se & McKeown[2]) has shown that the uterus will react in this way without t presence of a foetus and its trophoblast. The structure so formed is known as "deciduoma" and at its maximal size will fill the whole uterine lumen. A dec duoma can be produced during certain periods of lactation, but not duri pregnancy. In the normal sexual cycle the active secretion of progesterone fro the corpora lutea is necessary for the production of a deciduoma; oestron inhibits it. Deciduomata sometimes occur spontaneously, e.g. in animals on diet deficient in vitamin A (Bishop & Morgan).

Another reaction of the uterus is known as the "endometrial mole". Mil traumatisation of the uterine wall such as is requisite for the deciduoma leads t no specific reaction in the oestrone-treated ovariectomised rat, but if the uterin wall is slit, a tumour appears structurally resembling the hydatidiform mol occasionally spontaneously produced by the foetal part of the placenta (Sely Harlow & McKeown; Selye & Friedman). Oestrone is necessary for this reactio Hydatidiform moles contain large amounts of oestrone (Philipp), and these uterin tumour-like structures remind us of the connection between the sex-hormone and the carcinogens, to be referred to below (p. 253), especially as regards mammar cancer.

The general upshot of these findings, therefore, is that we must suppose th stimulus of the foetal trophoblast not to be very specific. Given the favourabl endocrine situation, the uterus will respond even to mechanical stimuli. Com petence (see p. 112) is the variable factor. It is interesting that in the later stages o a deciduoma there is even a breaking down of the endothelium of the matern blood-vessels, so that a blood sinus similar to that of the inter-villous space o the normal placenta is formed.

If we trace back the placenta to its first origins, we come, of course, to th formation of the foetal trophoblast by the expansion of the blastocyst. Th valuable investigations of Pincus and his collaborators on the expansion of th blastocyst* (review by Pincus[2]) have shown that this crucially important stage i mammalian development is under the control of a hormone secreted by th ternal organism, progesterone (see p. 252). The rabbit embryo, for example, ›ands from 0·12 mm. diameter in the Fallopian tube to nearly 8·0 mm. meter 6½ days later at the time of implantation. Corner[2] first found that in the ·ri of ovariectomised rabbits this expansion will not occur, and Pincus & ·rthessen[1] observed that when eggs are cultured *in vitro* it will not occur either, less progesterone and glutathione are both present. These substances prevent : collapse or herniation of the blastocyst and render its expansion nearly rmal. The oestrogens show both stimulatory and inhibitory effects, for which ıcus & Werthessen[2] have advanced explanations.

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* I am unable to understand why these authors persistently term this phenomenon "ovu growth"; a practice which calls to mind Napoleon's comment on the Holy Roman Empire.

1·91. Placental constitution

Practically every constituent substance for which search has been made in the ıcenta has been found there—carbohydrates, proteins, fats, enzymes,* vitans,† hormones,‡ etc. (cf. *CE*, Section 20). Yet we are still a long way from ving a complete account, even for the placenta of one single mammalian ecies, of the way in which its chemical constitution varies with the development the foetus.

Perhaps the most classical function of the placenta in this sense is its action a store of glycogen before the embryonic liver is in a position to take on its ycogenic function. This was known already in the middle of the last century rough the work of Claude Bernard (see *CE*, pp. 1019 ff.). It has recently been ıborated by Szendi[3,4], who brings forward evidence to show that there are, during e development of both rabbit and man, a succession of peaks of glycogenntent, first in the maternal part of the placenta, then in the foetal part, then in e foetal lung and lastly in the foetal liver. Szendi & Papp have also found that ycogen is richly contained in the histotrophe (the secretion of the uterine nds). That the placenta behaves much more like liver than muscle was well nonstrated by the experiments of Davy & Huggett, in which human placenta, autolysis, was found to produce practically no lactic acid but only glucose. some animals, such as the rat, the peak of glycogen storage must come quite ·ly in development, judging by the results of Corey[3] and Eufinger. In accordance :h earlier work, the placental glycogen seems to be insensitive to endocrine ıtrol, for Corey[4] found that adrenalin and insulin affected the glycogen-content both maternal and foetal liver, but not of the placenta.§

As regards the protein constitution of the placenta, there is little to add to ·mer accounts. Drage & Sandstrom, however, have shown that the amino-acid ;tribution in human placenta globulin is essentially the same as that in human ·um globulin, and Graff & Graff have disposed of the old story that placenta oteins were unusually rich in arginine.‖

E.g. deaminase, human (Abe[2]); kathepsin, human (Abe[2]); catalase, human (Bidone); nuclease, .man (Edlbacher & Kutscher); urease, human (Abe[2]); amylase, maltase, invertase, lipase, thepsin, rabbit (Abe[2]). Other enzymes will be referred to below, p. 89.

E.g. vitamin A, human (Gaehtgens; Dann[2]; Goldhammer & Kuen); vitamin C, human, histoemically (Tonutti & Plate), chemically (Neuweiler[1,2]).

E.g. thyroxin, human (da Re) and see below.

There is also histochemical work on glycogen in the placenta by Silvestri (rabbit) and Melandri d Szendi[1] (man).

See also Laufberger's work on amino-acids of the human placenta, and Tenney's on its collagen istol.).

Apart from histochemical studies of fats and lipoids in the placenta* there h been some valuable work. It is agreed (Boyd & Wilson; Effkeman) that t conveyance of fatty acids to the foetus is almost wholly in phosphatide for Towards the end of development in man, the daily utilisation of the foetus fro the blood is 35–40 gm. phosphatides, about 7 gm. each of free cholesterol ar cholesterol esters, and only 3 gm. of neutral fat. Starting from this point, Boy argued that if the placenta was acting purely as an organ of transmission i constitution should not change as the constitution of the embryo changed, b that if it were absorbing lipoidal materials and then secreting them in suitab proportions there should be a variation of this kind. The results of a careful stu showed that the lipoidal constitution of the rabbit placenta does indeed chan considerably, but not always parallel with the constitution of the foetus. We ma take the various fractions in order. In percentage phosphatides there is a stead rise in the foetal body, from about 100 to 1000 as embryo wet weight rises fro 1 to 51 gm., but in the placenta there is a marked fall, from 1600 to 110 followed by a rise parallel with that of the foetus, ending at 1500 mgm. %. Fo free cholesterol the picture was simpler, foetus rising from 50 to 150 mg. % an placenta from 200 to 300 mgm. %,† but for cholesterol esters the picture wa different. The foetal level remained constant at 25 mgm. %, but that of the placenta rose from 150 to a maximum at 400 and fell again to reach 150 at th end of development. This peak corresponded approximately in time to the fal in phosphatides already referred to, but it is by no means clear why at a certain time there should be a special tendency for fatty acids to be esterified by cholesterol. As for neutral fat, placenta and foetus behaved quite differently. The content of fat in the foetus rose rather suddenly towards the end of development from 200 to 1200 mgm. %, while the placenta showed a vaguely defined fall.‡ This interesting work is the only attempt so far made to correlate the constitution of placenta and embryo. It suggests that the placenta, though for many substances clearly acting purely as an ultra-filter, may in the case of certain water-insoluble substances have an important secretory part to play.

Arising out of the reference to phosphatides, it is of interest that choline, perhaps derived from them, and probably mainly in the form of acetylcholine. is contained in large amounts in the human placenta (Strack & Loeschke; Bischoff, Grab & Kapfhammer; Strack, Geissendorfer & Neubaur; Strack & Geissendorfer; Reynolds & Foster; Hauptstein). Indeed, according to Chang & Gaddum, human placenta is the richest source of choline in the body (28γ/gm.) with the exception of spleen. From the work of the Chinese school (H. C. Chang & A. Wong; Chang; Wong & Chang; Wen, Chang & Wong; Chang, Wen & Wong) we know that acetylcholine is derived wholly from the villous tissue of the foetal trophoblast and that the uterine muscle is certainly sensitive to it. That it plays a part in the muscular contractions of birth is probable enough, and acetylcholine may indeed be used as a stimulant to such contractions, which, unlike those caused by pituitrin, are rhythmical. In placentas from Caesarean sections the acetylcholine content is higher than normal. There appears to be a bound form of acetylcholine in the placenta.

As there seem to be only very small amounts of acetylcholine in the placentas of rodents, ungulates and carnivores, it seems likely that this mechanism has developed in special association with the villous haemo-chorial placenta, and it uld therefore be of much interest to know whether primates other than man ow it. In primitive primates with a labyrinthine placenta it should be absent. e placentas of rodents, carnivores and ungulates, however, contain choline.

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* E.g. Piccardo; Yamaguchi: see also fragmentary lipoid analyses by Corda; Jowett; and Klaus.

† This agrees with earlier work of J. Suzuki on the same animal.

‡ This contradicts earlier work of J. Suzuki, who found a rise, but the scatter is very large.

Of course, it is generally held that much more responsibility in the birth process to be ascribed to endocrine factors (such as the oxytocic hormone of the sterior pituitary) and to the increasing sensitivity (competence) of the uterine scles. Apart from reviews on this subject (Gibbons; Tapfer; Tapfer & slhofer), more or less illuminating, there is much interesting work by the ottish group, which will be found in the papers of Bell & Robson; Robson; bson & Bell; Robson & Schild, etc. but which cannot be described here—see o the papers of Allan & Wiles, Heller & Holtz, and Witherspoon. One of the ost interesting possibilities which arises out of this is that the foetus itself may ntribute to the initiation of birth, since in pigs and sheep, at any rate, the sterior pituitary at the end of development contains a considerable amount of e hormone. The quantity in the combined foetal glands at least equals that in e maternal gland. But what sensitises the uterine muscle to the hormone is as t unknown.

As Snyder[1] and many other workers have shown, birth may be inhibited for a nsiderable time if new corpora lutea are formed during the gestation period. For ample, if a rabbit is made to ovulate by a single injection of pregnancy urine tract on the 25th day, birth will fail to occur on the 32nd day as it should do, and ll only happen on the 40th day, i.e. at the time when the induced set of corpora tea degenerate. Under such conditions the uterus, though at full term, will not spond to pituitrin. Such experiments decisively contradict some old-established ews, connecting birth with changes in the foetus, senility of the placenta, echanical distention of the uterus, and the like. In such experiments it is also und that the life of the foetuses can continue until about the 36th day, but not nger; this fixes the reserve capacity of the placenta for supporting life.

Although the corpus luteum is essential for the formation of the placenta in mammals, its continuing presence throughout pregnancy is only necessary in ne (e.g. rabbit, mouse, opossum; not in guinea-pig, cat, man, horse and key). The same is true of the pituitary, but only because of its influence on ovary. Although the sexual cycle is in general obliterated during pregnancy ll mammals, traces of it may persist, and it appears that the periods of gestation nearly always simple multiples of the sexual period itself.

The question of what hormones exist in the placenta and whether the placenta elf has endocrine functions has attracted much attention. The extensive erature may be approached by way of several excellent reviews, such as those Clauberg; Collip, Thomson, Browne, McPhail & Williamson; Selye, Collip Thomson; Newton[2]; Corner[3]; and Snyder[2]. "It is now more or less taken for anted", writes Newton[2], "that the placenta is an endocrine organ, but its nction, except in the most general terms, is not understood....There is a ethora of data on the profound changes which take place during pregnancy, inting more and more to the placenta as a focus, but the unifying conception hich would make everything fall into place is elusive."

The presence of oestrogenic material in the placenta was adumbrated before e war (1913) by Aschner and others, but not proved (because the vaginal smear st was then not known) until 1924 by Doisy, Ralls, Allen & Johnston. Crystalline striol (see p. 254) has been isolated from the placenta, and the water-soluble strogenic principle discovered by the Montreal school ("emmenin") has turned t to be oestriol glucuronide. In actual amount the placenta contains at least as much and probably more oestrogenic material per gram than the ovary itse that the placenta actually secretes oestrogenic hormone depends on the in pretation of experiments in which the excretion of the hormone continues the secondary organs do not atrophy after the removal of the ovaries from pregnant organism. For detailed discussion of these points the reviews of Newt and Corner[3] must be consulted; Newton points out that the possibility contribution from the foetal gonads should not be overlooked. Neverthel there is strong presumptive evidence that the placenta does secrete oestroge hormone.

Progesterone, the corpus luteum hormone (see p. 252), has also been repeate demonstrated in the human placenta. That it is formed by the placenta appe quite probable. Progesterone seems to be excreted as pregnandiol glucurona and it is significant that this substance increases to its maximum excreti towards the end of pregnancy when the placenta is largest.

Our knowledge of the presence of hormones identical with or similar to th of the anterior pituitary in the placenta also begins with Aschner, who in 19 obtained ripening of Graafian follicles after injection of placenta extracts in young test animals. Since then the presence of something very like the gonad trophic hormone of the pituitary in the uterus has been firmly established, a there are many observations, detailed in Newton's review, which indicate th the placenta actually produces it (see also Philipp & Huber). Among the mo interesting of these are the experiments of Kido[2], who was able to cultivate huma placental tissue in the eye-cavity of the rabbit, and found later that the gonad trophic hormone was circulating in the blood and excreted in the urine. Nagayam and Gey, Seegar & Hellman also claim to have demonstrated the synthesis both oestrogen and gonadotrophic hormone by placental tissue growing *in vitr* On the other hand, there are a number of indications that the gonadotroph substance of the placenta is not exactly the same as that liberated by the pituitar and it seems to be unable, even in the largest doses, fully to replace the hormo of an animal's own pituitary. This is often thought to be due to the placen containing only the luteinising and not both the luteinising and the follicl stimulating components of the gonadotrophic hormone, but the real state affairs is probably not so simple. Glebova has found the pituitary "ketogen hormone" in the human placenta.

Finally, androgenic substances have also been found in the human placen (Goecke, Wirz & Daners), and extracts of it are said to accelerate amphibia metamorphosis (Ganfini).

In endocrinological complexity, therefore, the placenta seems to outdo eve the pituitary, and it will probably be a long time before we have a satisfactor understanding of its many functions. It will in particular be interesting to tra down the production of the various hormones from the evolutionary point view in order to gain some idea of how the extraordinary complexity of th chorio-amniotic villous haemo-chorial placenta of man arose. A good beginnin in this direction has been made by Glebova.

Among the other substances present in the placenta† the pigments are notabl but discussion of them will be postponed till later (see p. 647).

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* Though Parker & Tenney show that the total oestrogen in both foetus and maternal organis exceeds that in the placenta.

† [Descriptive bibliography by L. Zuntz[1].] Glutathione, human (Macciotta); total ash, rabb (Furuhashi); iron, human, histochemically (Mayeda; Šesler; Roussel & Deflandre[1]), chemical (Hilgenberg). The massive lime deposits in the placentas of ungulates (Höpping) are noteworth

ike the yolk-sac of the chick (p. 70), the mammalian placenta may continue e for some time after the death of the embryo to which it belongs. In the use (Newton[1]), and the monkey (G. van Wagenen & Newton) the placentas of uses dead or removed are delivered later on at the end of the normal gestation iod. Placenta alone is thus quite able to suppress the oestrous cycle. Brooksby Newton have also shown that the placenta exercises an influence on water ntion by the maternal organism, there being a sharp drop in weight when the centas are delivered. This is not without interest in view of what has already n said about the water relations of terrestrial animals (p. 33), and the loss mniotic liquid at birth by most mammals is small.

1·92. Placental metabolism

Much less attention has been paid to the metabolism of the placenta itself than he hormones in it or to the transmission of substances through it. In continua- n of the observations discussed in *CE*, p. 1461, Loeser has made manometric imations of the metabolism of the human placenta, with the following average ults:

	Q_{O_2}	$Q_L^{N_2}$	$Q_L^{O_2}$
1st month	—	+17·6	—
2nd month	−5·0	+ 7·0	+5·1
At term	−1·4	+ 3·5	+0·57

idently, like all other ageing tissues, the placenta suffers a decline of metabolic ivity, but it is interesting, in view of the invasive activities of the trophoblast, often suspected as resembling malignant tissue, that there should be an aerobic colysis of appreciable extent at the earlier stages. Substantially the same res were obtained for rabbit and cat placenta. The effects of glucose, adrenalin, emia, etc. on these levels have been studied by Matsuhita, but not with very king results.

The enzymic equipment of the placenta is not at first sight interesting, ce practically every enzyme which has been looked for in the placenta has n found there (review by Mayer & Seitz, as well as *CE*, pp. 1481 ff.). The ent trend in this work is the study of the dehydrogenases, which have been ind for succinate (Thunberg; Tesauro), fumarate (da Cunha & Jacobsohn) d glycerophosphate, lactate, citrate, glycerate, glutamate, hexose-diphosphate, . (Thunberg). Weak phosphatases attacking diphenyl-pyrophosphate (da nha) and phosphoglycerate (Antoniani & Clerici) are also present. Particularly eresting is the fact established by Busse that the phosphatase of placenta acking glycerophosphate is more active than that of any adult organ and creases in strength during foetal development. Correspondingly, Busse could ver get an esterification of phosphate in the placenta under any conditions. also found that the zymohexase of placenta (the enzyme converting hexose- phosphate to triosephosphate) was extraordinarily weak, placenta having only $\frac{}{00}$th the activity of muscle. Yet when Kutscher, Veith & Sarreither examined lactic acid production of placenta and muscle under identical conditions, that of the former was found to have only $\frac{1}{5}$th the activity of the latter.* Pasteur reaction is not very marked in placenta.

An active deaminase has been found in placenta by Botella-Llusia[2]. As plac produces urea from ammonium carbonate during autolysis (Botella-Llusia[1]) may surmise that the ornithine cycle is functional there, though this has no yet been directly proved.

1·93. Placental transmission

From the former discussion (*CE*, Section 21) four main points emerg (1) There is a relationship between placental transmission and molecu size, smaller molecules penetrating through the placental barrier more read than larger ones, as through an ultra-filter. (2) In this respect the placen of different mammals differ, mainly in accordance with the number of c layers interposed between the foetal and maternal bloods. Probably th are also considerable differences between the behaviour of the placentas of t same mammalian species at different times in development. (3) Transie increases in the concentration of a diffusible substance on one side of t barrier lead to corresponding increases on the other side. (4) In spite of t fact that the placental barrier behaves like an ultra-filter, there are persiste differences in the levels of the various constituents of maternal and foet bloods which it is at present impossible to account for either by the Donna or any other similar theory, but which in all probability have their explanati in physical mechanisms of the kind postulated by such theories. All the wo which has been done since 1931 bears out these statements.

On the whole subject of placental transmission there are valuable reviews b Schlossmann[3]; Runge; and F. J. Koch, besides the descriptive bibliography L. Zuntz[3]. Taking first the properties of the placental barrier as an ultra-filte the old conclusion that discrete particles never pass through unless, like pathogen bacteria, they make lesions in the placenta, has been further substantiat (Nakagawa; Szendi[2]). Non-pathogenic bacteria never penetrate; colloidal pa ticles of intermediate size may or may not. Some phages penetrate (Nakagaw while others cannot do so (Natan-Larrier, Eliava & Richard). As has alrea been noted (p. 30), antigenic proteins in general do not go through but ant bodies do, hence the passive immunity of the foetus. A good example of this lactalbumen and its antibody (guinea-pig; Natan-Larrier & Grimard-Richard There are exceptions to this, however, e.g. ovoalbumen, which, according Natan-Larrier & Richard[1], can pass through the guinea-pig placenta. There is curious effect of soaps discovered by these investigators; horse-serum protei will penetrate the guinea-pig placenta, normally impermeable to them, if quantity of sodium oleate, which may be very small, is injected first (Nata Larrier & Richard; Natan-Larrier, Noyer & Richard). The same effect w obtained by Natan-Larrier & Grimard-Richard[2] with bile salts and ov albumen, but no direct relation could be found between the fall in surfa tension of the blood caused by these substances, and their effect on the placent permeability.

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* A non-phosphorylating path for carbohydrate breakdown in placenta is therefore indicated (s p. 610).

)f greater interest than the numerous positive or negative observations on
:ental permeability to one or another substance* are the experiments which
w progressive changes in permeability during foetal development (cf. the
k of Rodolfo, already mentioned, p. 31). Thus Lell, Liber & Snyder, using
nolsulphonephthalein, found that the rabbit placenta was much more
meable in the foetal → maternal direction at the 22nd than at the 29th day,
conversely the leakage into the maternal circulation from the amniotic liquid
much greater at the 30th than at the 21st. Similar changes have been
nd by a number of investigators, e.g. Snyder & Speert showed that the rate
:ransmission of neoarsphenamine from maternal to foetal circulation in the
bit greatly increases during development. The As content of the foetus 24 hr.
r the injection rises from less than 1 mgm. AsO_3 at the 19th day to 20 mgm.
erm. In the rat, too, according to Corey[1], placental permeability to insulin is
atly increased towards the end of the developmental period. The placentas of
bit, guinea-pig and dog, according to Birguer & Afanasiev, become pro-
ssively less permeable to salts of bromine and iodine as development goes on.
ere is here a wide field for future work.

'assing now to the concentration differences present in the two blood streams,†
nay in general be said that the maternal blood is richer in total solids, proteins,
ıl P, lipoid P, phosphatides, neutral fat, cholesterol, and glucose; while the
tal blood is richer in non-protein N, free amino-N, inorganic P and calcium.

'he following data, additional to those in *CE*, Section 21, may be found useful:
ı) *Man:* amino-acids + (Bickenbach & Rupp); dipeptides + (Bickenbach & Rupp); glucose +
e[3]); phenoisulphonephthalein + (Albano); citrate + (Lenner); oestrone − (Levy-Solal,
lther & Dalsace); barbiturates + (Berutti); lead + (Baumann); arsenic − (Dejust & Vignes;
ès); arsphenamine + (Eastman & Dippel); various metals and metalloid elements (Farès).
2) *Dog:* amino-acids + (Schlossmann[2]); insulin + (Schlossmann[4]); barbiturates + (Fabre);
ıine + (Regnier); parathyroid hormone − (Hoskins & Snyder); thyreotrophic hormone of
ıitary + (Döderlein).
) *Rat:* glucose + (Corey[1]); thyroxin and thyrotrophic hormone of pituitary + (Thérèsa);
in + (Corey[1]); adrenalin + (Corey[4]; Ingle & Fisher); androgens and oestrone + (Hain);
olsulphonephthalein + (Boucek & Renton); fluorine + (M. M. Murray); colloidal thorium
ıde − (Menville & Ané); radioactive sodium + (Flexner & Pohl; Flexner & Roberts).
) *Mouse:* bismuth and antimony − (Lebedeva).
) *Rabbit:* glucose, pentoses + (Brandstrup[1]); sucrose, lactose − (Brandstrup[1]); amino-acids +
ndstrup[2]); oestrone + (Skowron & Skarzyński); adrenalin, posterior pituitary, acetylcholine
mine + (Cattaneo[3]; Falaschino); nicotine + (Sergueev).
) *Goat:* glucose + (Passmore & Schlossmann); adrenalin + (Schlossmann[1]).

he following succinct summary of the most recent quantitative work may be of interest;
ıe concentration in the foetal blood, $m \equiv$ that in the maternal blood, $c \equiv$ confirmation of earlier
summarised in *CE*, Table 243, $nc \equiv$ contradiction with it. There are some general figures
ıe review of de Toni.
) *Human* (nearly always at term): total solids, $m > f$ (c) (Levy-Solal, Dalsace & Gutman;
man & Levy-Solal; Naeslund[1]); total protein N, $m > f$ (c) (Levy-Solal, Dalsace & Gutman;
tman & Levy-Solal; Naeslund[1]; Pommerenke; Puccioni, de Niederhausen & Roncallo);
inogen, $m > f$ (Naeslund[2]); non-protein N, $m < f$ (c) (Naeslund[1]; Pommerenke); amino-acid N,
$= f$ (nc) (Doneddu), $m < f$ (c) (Naeslund[1]; Pommerenke; Legrand); polypeptides, $m < f$ (Le-
nd); glucose, $m > f$ (c) (Levy-Solal, Dalsace & Gutman; Gutman & Levy-Solal); citrate,
f (Lenner); choline, $m > f$ (Späth); vitamin A, $m > f$ (Gaehtgens); vitamin C, $m < f$ (Wahren
Rundquist; Manahan & Eastman); neutral fat, $m > f$ (c) (Levy-Solal, Dalsace & Gutman;
man & Levy-Solal); cholesterol, $m > f$ (c) (Rosenbloom); carotin, $m > f$ (Gaehtgens); creatinine,
f (c) (Naeslund[1]); uric acid, $m = f$ (c) (Naeslund[1]); lactic acid, $m = f$ (nc) (Eastman & McLane);
athione, $m < f$ (Anselmino & Hoffman[1]; Lemeland & Deletang; Buzzi; Oberst & Woods;
ı); fumaric dehydrogenase, $m > f$ (da Cunha & Jacobsohn); choline esterase, $m > f$ (Navratil);
lase, $m < f$ (Anselmino & Hoffman[1]); ash, $m < f$ (Levy-Solal, Dalsace & Gutman; Gutman &
-Solal); total base (Thompson & Pommerenke); calcium, $m < f$ (c) (Garofalo; Mull; Otte);
li reserve, $m > f$ (Puccioni, de Niederhausen & Roncallo); total P, $m < f$ (Mull); inorganic P,

There are certain exceptions to this; for example, in ungulates (epithelio-chor placentas) the blood-sugar is higher in the foetal than in the maternal circulatic The concentrations of urea, uric acid, creatine, creatinine, chloride, etc. a usually the same. The differences of concentration are not the only differenc between the bloods. Thus the foetal blood in man is richer in haemoglob (Börner) and in red blood corpuscles (Lucas & Dearing; Seckel), a fact whi probably accounts for the higher blood glutathione on the foetal side (Lemelar & Deletang; Oberst & Woods). The surface tension of the foetal blood is co siderably higher than the maternal (Kisch & Remertz) and the sedimentati rate of red blood corpuscles differs (Bogaert; Puccioni, de Niederhausen Roncallo), being ten times faster on the maternal side. This phenomenon is du to the plasma, for Bogaert found that maternal red blood corpuscles in foet plasma are slowed down, while foetal red blood corpuscles in maternal plasm are accelerated to the maternal rate. The colloidal osmotic pressure of foetal an maternal sera in the sheep differs (McCarthy), for the foetal serum has mo albumen and exerts a 50% higher osmotic pressure per gram protein. Th cause of this difference is not understood. The plasma protein continues t rise before and after birth (Clark[1]; Clark & Holling; Swanson & Smith) mor or less parallel with the blood pressure. The very low fibrinogen content of foeta blood explains the occasional occurrence of temporary *haemophilia neonatorun* (Naeslund[2]).

There can be little doubt that an explanation of the same type as the Donnar equilibrium, but perhaps more complicated, will eventually be found to hol good for the placental barrier.* Luck & Ritter, in suggestive experiments, foun that if a cellophane membrane is set up with a protein solution on one side an a solution of amino-acids on the other, neither osmotic equilibrium nor Donnar equilibrium is ever attained, even at the isoelectric point of the protein.† It i nevertheless striking to see, as in the graphs published by Schlossmann[2] an Passmore & Schlossmann, a large rise in the amino-nitrogen-content of bot bloods in the dog, or of the glucose-content of both bloods in the goat or sheep upon injection of the given substance into the maternal circulation, after which

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$m<f$ (c) (Timpe); organic P, $m>f$ (c) (Timpe); oestrone, $m<f$ (Siegert & Neumann); pituitrin $m=f$ (Siegert & Neumann).

(2) *Cow:* total protein N, $m>f$ (c) (Sánta); non-protein N, $m<f$ (c) (Sánta); fibrinogen, $m>$ (Sánta).

(3) *Pig:* non-protein N, $m>f$ (Dobó).

(4) *Rabbit:* amino-acid N, $m<f$ (c) (Brandstrup[2]).

(5) *Dog:* amino-acid N, $m<f$ (c) (Schlossmann).

(6) *Rat:* plasma phosphatase, $m>f$ (Weil).

(7) *Mouse:* oestrone, $m=f$ (Soule).

* Bickenbach & Rupp[3] believe, however, that the excess of amino-nitrogen in the foetal blood i only an apparent phenomenon due to the fact that the maternal blood with which the foetal bloo has usually been compared is venous. On comparing the foetal level with the maternal arteria level the difference was reduced or abolished. Nevertheless, it is very doubtful whether a explanation of this kind can hold good for all the constituents which have been found to be mor concentrated in the foetal blood.

† It is interesting to compare the placental barrier with other barriers such as the haemato-encephalic barrier. The formation of the cerebro-spinal fluid in the pig foetus has been studie by Flexner. At first it is in Donnan equilibrium with the plasma, but rather suddenly its composition changes, whether as a result of secretion or as the result of a new form of equilibrium i not clear. Flexner & Stiehler went on to show that this change-over is accompanied by an increas of dehydrogenase and cytochrome oxidase activity, the appearance of a potential difference, etc in the barrier (the choroid plexus) itself.

the concentrations on both sides of the barrier settle down at about their original levels, but with the foetal side still some 5 mgm. % *higher* than the maternal.

Some ingenious experiments have been made by Bickenbach & Rupp[1] to study the passage of nutrient substances through the placenta. They found, for instance, that if methyl or amyl oleate or palmitate were injected into the maternal circulation (in man), these unusual "glycerides" were found again on the foetal side, and concluded that if there is a hydrolysis at the barrier followed by a synthesis, it must take place so fast that there was no chance whatever of the placenta being able to form normal triglycerides instead of these esters. Or again[4], on injecting brominated serum proteins into the maternal circulation in rabbits, no trace of bromine was ever found in the foetal circulation or tissues, indicating that the serum proteins of the maternal blood are not in danger of being hydrolysed by the placenta to furnish amino-acids for the foetus. Dipeptides pass through without being hydrolysed.

1·94. The amniotic and allantoic liquids

The amniotic liquid of a mammalian species still awaits an exhaustive examination. The data formerly described (*CE*, Section 22) were all of a somewhat unsatisfactory kind, partly because of the use of estimation methods which would hardly command reliance to-day, and partly because of the extremely large statistical variations naturally occurring in the composition of the liquids. The only recent examination of the osmotic pressure, for example, of the amniotic liquid is that of Howard[3], who believes that it approximates to that of the serum in the pig and man (Δ −0·55°), while that of the allantoic liquid is much lower (Δ −0·20°). But as doubt had to be thrown in another connection on the validity of freezing-points taken with this author's methods (see p. 14), we are not much further advanced than we were in 1931. According to Makepeace, Fremont-Smith, Dailey & Carroll the osmotic pressure of the amniotic liquid in man at term is a little below that of the serum. Good exact measurements of the volume of the amniotic liquid in the rabbit are given by Lell. It declines markedly towards the end of gestation, and there is therefore little water-loss at birth (cf. above, p. 88). This decline may even be found in man (Dieckmann & Davis). Snyder & Rosenfeld and others have believed that the amniotic liquid normally enters the foetal lungs in the early respiratory movements, but it is now fairly certain that this never occurs under normal physiological conditions (Windle, Becker, Barth & Schulz), though swallowing of amniotic liquid does. The amnion itself is furnished with muscle fibres, which like those of the chick embryo's amnion (Pierce; Kuo[1]; Revoutskaia) produce long-continuing contractions (Groebbels[3]). Data for the gas pressures in the amniotic liquid of the rabbit have been given by Campbell, and many substances have been found to be present in it.* In man, for example, urea and uric acid accumulate to a slight extent (Guthmann & May; Cantarov, Stuckert & Davis).†

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* E.g. for *Man* [descriptive bibliography by L. Zuntz[2]]: general composition (de Laurentis; Makepeace, Fremont-Smith, Dailey & Carroll; Cantarov, Stuckert & Davies; Shrewsbury); glucose (Mohs); glutathione (Guercia); citrate (Genell); erepsin (Abe[4]); adrenalin (Macchiarulo); myotonic and vasopressor substances (Fomina); thyroxin (Contardo); anterior pituitary hormones (Huddleston & Whitehead; Cozzi); oestrone (Loewe; Morrell, Powers & Varley); for the merino *sheep*: general composition (Malan, Malan & Curson).

† A few observations on the liquids of the chick embryo will be found referred to on pp. 606, 662.

Part Two

THE MORPHOGENETIC STIMULI

"Die beschreibende Chemie der lebenden Teile kann somit als eine Fortsetzung der Morpholog in das Reich der kleinen Dimensionen angesehen werden, und es ist wohl der Gedanke berechtigt, beide Wissenszweige mit denselben geistigen Hilfsmittel in zusammenfassender Weise z bearbeiten."

A. Kossel, 1921

"The problem of (hereditary) transmission might be merged in the broader problem of th production of form through chemical processes—the central problem of all development."

E. S. Russell, 1916

THE MORPHOGENETIC STIMULI

2·05. Introduction.

Although our knowledge of the morphogenetic stimuli which operate from one part of the embryo to another during development is relatively new, it has profoundly modified our picture of the mechanism of the developmental process. So important, indeed, are these modifications that an apology at the outset can hardly be dispensed with on the part of one whose training was not morphological and whose only merit is a genuine effort to pass from the familiar ground of chemistry to the difficult country of the morphologist, bringing what help he can.

A discussion such as the following may be modestly compared to one of those narrow-gauge railway lines joining distant places, in which some European countries abound. Sometimes it runs alongside the well-polished tracks of trunk communications, sometimes it strikes across country, winding through pastoral or wooded valleys with more originality; at one point it touches some obscure but charming village, at another it pauses for a moment to make a junction with a main road. In the present state of our knowledge this is all that the biochemist can contribute, and it must emphatically be stated that the following discussion is not intended to replace in any way the consultation of the works of modern experimental embryologists, classical in some cases already. The "chemin-de-fer vicinal" must not be mistaken for the routes of the great expresses.

To these a reference must first be made. The most important event since 1931 is the publication of Hans Spemann's Silliman Lectures[17] at Yale University, in which he has given us an unparalleled account of the principal processes in development, though confined to the development of amphibia. Covering a wider field is the excellent book of Huxley & de Beer[2], which has the advantage of a continuity with the older theories of the American schools.* A different advantage is possessed by the monograph of Dalcq[4], namely an acquaintance at first hand with the phenomena of development in ascidians and hence an attempt to link together all the chordates in a logical scheme. The little book of Weiss[11], with its stimulating discussion of regeneration phenomena,† has somewhat aged, but he has now supplemented it with his valuable *Principles of Development*. The massive compilation of Schleip remains the most useful account of the comparative aspect of experimental embryology, while Albert Brachet's great text-book has been well revised and brought up to date by Dalcq & Gérard. Finally, the border-line between embryology and genetics has been exhaustively surveyed by Goldschmidt[8] in the third incarnation of his classical essay. Books of les importance, though not without value, are those of Dürken[2] and May[2]. The v mass of data in Korschelt & Heider has been conveniently summarised Richards[2].

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* It has also the disadvantage, however, that all the phenomena of development are forced rather artificially into the framework of the gradient theory. Examples from invertebrates and vertebrates are placed side by side to illustrate principles not yet themselves fully established, so that the result is sometimes confusing. Though admiring the synthetic genius of these authors, I do not feel that the subject can as yet be so clearly systematised, and I shall not attempt the like here.

† To be received with caution, however; see p. 435.

A number of smaller monographs should also be mentioned. The m modern is that of Dalcq[7], which adumbrates a comparative experimental, n merely a comparative, embryology, and contains an account of the double-fi theory (see p. 220). The most theoretical is that of Bertalanffy & Woodger, whi gives a critical review of modern theories of development, and there are chara teristically stimulating books by H. Przibram[6] and N. K. Koltzov[3]. Monograp on the experimental embryology of special groups have hardly yet begun appear, though it is hard to refuse this designation to the inaugural thesis Lindahl[7] on the development of echinoderms. Poulson's monograph[2] on inse development is almost entirely descriptive, as are the contributions of Pincus[1] ar of Fischel on that of mammals. In the experimental embryology of special orga systems the nervous system has received most attention, in the books of Coghi and Detwiler, but there is also Murray's on the development of bones. Th monograph of Hatt on cell streams (morphogenetic movements) in early develop ment was valuable. Finally, there are the interesting contributions of J. Brachet[1] and of Waddington[26] on the role of the nucleus in early development.

We shall have occasion below to mention many of these monographs i specific connections.* In the meantime it must be emphasised that none of th sections of this book dealing with experimental morphology are to be taken a exhaustive. Journals such as the *Archiv. f. Entwicklungsmechanik* and othe similar publications contain a vast wealth of interesting facts. We shall only b able to consider such of them as allow of discussion along biochemical lines.

2·1. General concepts of causal morphology

There are two ways of penetrating into such a subject as this. The experimenta facts may be described in some more or less arbitrary order, with pauses from time to time to define the various concepts to which the facts have given rise Or, on the other hand, the concepts themselves may first be defined, and th experimental facts later marshalled in the light of these. Our knowledge o morphogenetic stimuli has now, it seems, advanced to such a point that thi second, more logical, way of proceeding may be adopted. A little repetition may however, be unavoidable, as we shall meet with some facts twice over, first i their theoretical connection, and then again as nodes in the factual network.

It is necessary to begin with the concept of *Determination*.

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* Apart from reviews mentioned later on, general discussions from which much can still be learn (though some, owing to later advances, have now to be read with caution) are to be found in the following: 1923, Spemann[11] (the historical connection with Weismann); 1925, O. Mangold[2] 1926, Przibram[4] (gives the background to the experiment of Spemann & H. Mangold); 1928 O. Mangold[4, 5]; 1929, Gilchrist[2] (caution); 1933, Harrison[12] (caution); 1934, Spemann[15]; Stolte (caution); Ermakov[1] (in Russian, caution); 1935, Weiss[14]; Huxley[11] (caution); Schmidt[2] (i Russian); 1936, May[4] (caution); Caullery[2]; Poležaiev[7] (in Russian); O. Mangold[14, 15]; 1937 Harrison[17]; Brøndsted[2] (caution); Daniel[2]; 1938, R. S. Lillie[2]; Poležaiev[9] (in Russian); Borovańsk (in Czech); 1939, Caullery[3]; Panagiotou (in Greek); 1940, F. E. Lehmann[16] (caution).

2·11. Determination

At the outset we meet with the contributions of Hans Driesch to the theory of morphogenesis. Although his theory of entelechy is now only a matter of historical interest (see p. 119), the investigations which led to it were quite fundamental. We are so accustomed in modern embryology to the concept of determination, i.e. the fixing of the fates of parts of the embryo at a definite time in development, that it is difficult to think back into the state of mind in the last century when it was supposed that fates were all fixed to begin with.

In 1883 W. Roux[1] enunciated the view that development was brought about by a qualitative division of the germ-plasm contained in the nucleus, and that the complicated process of mitotic division was primarily devoted to that end. It was thought that development proceeded by a mosaic-like distribution of potencies to the cells in segmentation, and that for instance the first cleavage furrow separated the material and the potencies of the right side from those of the left. In 1888 Roux[2] announced that, if one of the two first blastomeres of a frog egg was killed by cautery with a hot needle, a half-embryo developed from the uninjured cell. This "mosaic" development seemed to confirm the view of Weismann that the fates of all the parts were fixed at the outset, and, although Roux's particular experiment afterwards turned out to be deceptive, in that the amphibian blastomeres are not necessarily mosaic, a large number of other eggs were subsequently found in which any injury to the uncleaved or cleaving ovum is reflected in injuries or losses in the finished embryo.*

Considerable astonishment was therefore caused by Driesch's announcement[1] in 1891 that he had obtained complete larvae from single blastomeres of the sea-urchin egg isolated at the two-cell stage.† We may give his account of the discovery in his own words:

"The development of our *Echinus* proceeds rather rapidly, the cleavage being complete in about fifteen hours. I quickly noticed on the evening of the first day of the experiment, when the half-embryo was composed of about two hundred cells, that the margin of the hemispherical embryo bent together a little, as if it were about to form a whole sphere of smaller size, and indeed the next morning there was a whole diminutive blastula swimming about. I was so much convinced that I should get the Roux effect in all its features that, even in spite of this whole blastula, I now expected that the next morning would reveal to me the half-organisation of the subject once more; the intestine, I supposed, would come out on one side of it, as a half-tube, and the mesodermal ring might be a half also. But things turned out as they were bound to do, and not as I had expect there was a typically *whole* gastrula in my dish the next morning, differing fr a normal one only by its smaller size, and this small but whole gastrula followed by a whole and typical small *pluteus* larva.”

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* For a good account of the early work in experimental embryology, see E. S. Russell's admirable history of nineteenth-century biology, *Form and Function*. Mosaic development was described for ascidian eggs by Chabry in 1887 and Conklin[1] in 1905, for ctenophore eggs by Chun in 1892 and Yatsu in 1912, for molluscan eggs by Crampton in 1896 and Wilson[2] in 1904, etc. etc. It has been usual to express the difference between mosaic eggs and regulation eggs by saying that in the former all the determinative processes have been completed before fertilisation, while in the latter they extend throughout development, centering on gastrulation in the amphibian, for example, but lasting on even into the metamorphosis period. The most complete discussion of this is to be found in E. B. Wilson's[3] contribution to the Spemann Festschrift and in Weiss' *Entwicklungsphysiologie*, pp. 23 ff. Dalcq, however, in his recent book *Form and Causality*, shows that the distinction cannot be considered absolute, as minor degrees of regulation appear to be possible in mosaic eggs (see on, p. 325).

† v. Baer's account[5] of his study of echinoderm development in Trieste in 1847 is well worth hunting out.

In the following year[2] he showed that whole embryos could be produced fr one or more blastomeres isolated at the four-cell stage, and later that alterat of cleavage by compression[5], the fusion of two eggs into one[3], or the cutting certain directions of the original egg-cell[4], all permitted of normal embryos be produced.

Driesch[7] introduced the term “Prospective Significance” (*prospektive Bedeutu* to indicate the actual fate of any part or monad in the original egg. His gr discovery lay in the finding that the significance of such a part was not exhaust by its prospective significance, but that it was widely changeable according various circumstances. And he introduced the term “Prospective Potency (*prospektive Potenz*) in order to signify the collection of possible fates of such part. Thus the actual fate (the prospective significance) is chosen from among th possible fates (the prospective potency);* in normal development it will be on constant result, in abnormal development it may be quite a different one. Thi condition of multiple potency of the parts of the early egg-cell has been terme *Pluripotency*.

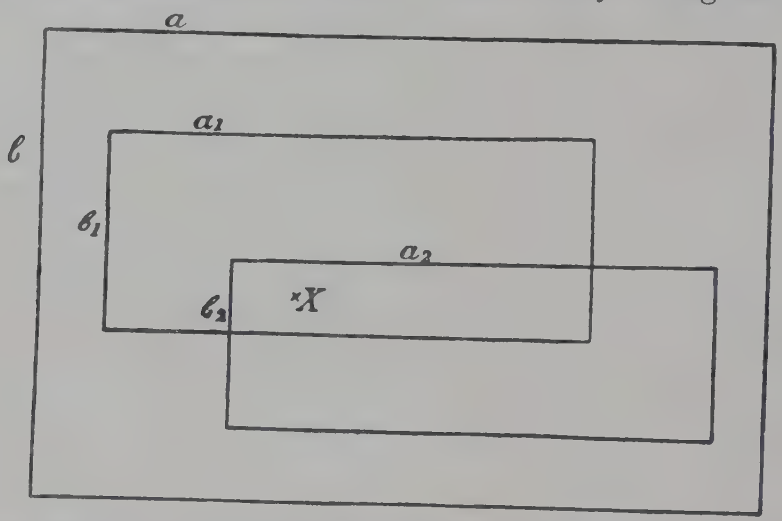

Fig. 37. Driesch's diagram to show the characteristics of a harmonious equipotential system.

These relationships were illustrated by Driesch[7] in the following way (Fig. 37) A plane of the dimensions *a* and *b* represents normal undisturbed development; taking the sides of the plane as fixed localities for orientation, we can say that the actual fate (the prospective significance) of every element of the plane stands in a fixed and definite relation to the length of the two co-ordinates at ight angles. Thus the point X would normally develop into some definite part f the finished embryo, e.g. eye-cup or muscle-cell. But its prospective significance s not as wide as its prospective potency, so that if it formed part of an isolated ortion a_1b_1 its relation to the whole would be different and its actual fate would e different. The same applies to its position in another possible isolated portion $_2b_2$. These isolated portions, it is to be remembered, regulate themselves in such way as to reproduce on a smaller scale the relative location of parts seen in the bsolutely normal case. They possess, therefore, a non-variable factor of wholeess, represented in Driesch's terminology by the symbol E.[*] In view of these roperties, systems which fulfilled the conditions just laid down for the plane *ab* ere called by Driesch "harmonious equipotential systems".

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* The concept of potency has been recently examined by Raven[5], who thinks that the word ha frequently been misunderstood since “potency” often means “power” rather than “possibility”. But the Aristotelian distinction between *potentia* and *actualitas* was the origin of the embryological use of the term; cf. “Parts of machines while at rest have a sort of potentiality of motion in them, and when any external force puts the first of them in motion, immediately the next is moved in actuality” (*De Gen. An.* 734 b, and cf. Ross' commentary on Aristotle's *Metaphysics*, pp. cxxiv ff.). Raven's other difficulties are, I think, avoided by a careful use of the concepts of competence and induction (see pp. 112 and 104).

We realise now, after forty years of experimental research,[†] that at the beginning of development, parts or monads of the egg, similar to that which has been represented by X in the diagram, are indeed undetermined,[‡] and that one of the most fundamental processes in development consists in the closing of doors, .e. in determination, in the progressive restriction of the possible fates.

By his brilliant extensions of Born's transplantation method,[§] Spemann[8] showed n the case of the newt that up to a certain stage of gastrulation the fates of most of the embryonic regions are not irrevocably determined. A piece of presumptive neural tube material removed from one embryo and grafted into another will turn into external gills if it happens to be grafted into the presumptive gill region of the latter. On the other hand, a piece of presumptive skin, if grafted into a suitable region of the presumptive nerve tube of a second embryo, will in due course turn into brain or spinal cord. Up to this stage in gastrulation, therefore, the regions develop always in step with their actual surroundings and without reference to their former surroundings, or their prospective significance if they ad not been interfered with. The German word "*ortsgemäss*" was coined to xpress this. The parts are extremely plastic, like the parts in Driesch's searchin eggs, which would obediently form gut or skeleton according to their osition in the whole. Nor does this plasticity or pluripotency stop short at the

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Driesch[7], p. 91. [†] Summarised in Huxley & de Beer's book. [‡] See glossary, Determination.

Though Gustav Born first discovered the healing powers of amphibian embryos and invented uch of the technique of operating upon them, he did not, owing to his early death, follow the ossibilities very far. Otto Mangold said to me once "er hatte kein Problem". Spemann's chievements have lain as much in the asking of certain questions as in the answering f them. Grafting and transplantation as such have a long history, but because easier in plants han animals, and in large organisms than in small embryos, did not quickly lead to fundamental iscoveries. Skin transplantations in man were known already in the sixteenth century. The ollowing passage strikingly illustrates how nearly the concepts of *ortsgemäss* and *herkunftsgemäss* were approached, though set in a frame of factual error. "A certain inhabitant of Bruxels, in a combat had his nose mowed off, and addressed himself to Tagliacozzus, a famous chirurgeon, living at Bononia, that he might procure a new one; and when he feared the incision of his own arm, he hired a porter to admit it, out of whose arm, having first given the reward agreed upon, at ength he digged a new nose. About thirteen moneths after his return to his own countrey, on a udden the ingrafted nose grew cold, putrefied, and in a few days, dropt off. To those of his riends, that were curious in the exploration of this unexpected misfortune, it was discovered, hat the porter expired near about the same punctilio of time, wherein the nose grew frigid and adaverous" (Walter Charleton, "A Ternary of Paradoxes", London, 1650). Evidently men ould speculate on the extent to which a piece of tissue transferred from one body to another, ould retain something of its individuality. Autoplastic transplantations appear to go a long way ack in the history of surgery beyond Gasparo Tagliacozzi (1546–1599) who brought them to a igh level (see Corradi). I owe this interesting reference to my friend Dr Charles Singer.

germ-layers themselves, for mesoderm and ectoderm, for example, are perfe interchangeable, as O. Mangold[1] demonstrated.

The process of gastrulation, however, is critical, for at this time the orig plasticity is lost, and the main fates of the parts are determined. This is irrevoca The early period of plasticity is followed by one of rigidity, each part be willing now only to undergo a certain special type of development which dif from part to part. If it is grafted into another embryo, it will continue differentiate in accordance with its inner determination, and not in accorda with the new situation in which it has been artificially placed. In other wor it will develop "*herkunftsgemäss*". The regions can now only develop towa their presumptive fates. Prospective potency* has been ruthlessly curtailed prospective significance. The eye region will form an eye whether this poi outwards to the external world or inwards to the body-cavity. The invisi process of determination has ushered in the new period of self-differentiatio in which the embryo has become a mosaic of irreplaceable regions, similar to th whole development of certain eggs ("mosaic eggs"), which never manifest period of plasticity or pluripotency.

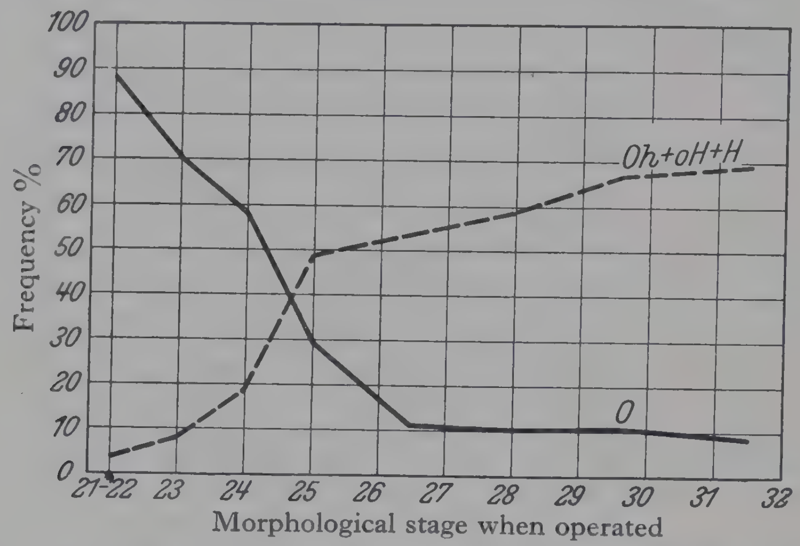

Fig. 38. Determination of the choroidal fissure of the amphibian eye-cup.

As an illustrative case of a statistically well-defined transition from neighbou wise to selfwise behaviour, we might choose the relatively unimportant choroid fissure which appears in the lower part of the amphibian eye-cup. T. Sato extirpated the eye-cup at an early stage, turned it upside down and put it bac As Fig. 38 shows, the number of cases showing neighbourwise behaviour, i.e. th appearance of the fissure at the lower part after this treatment, is approximatel the reciprocal of those showing selfwise behaviour, i.e. the appearance of th fissure at the "wrong" upper part after this treatment. Determination has thu set in at stage 23 and is complete at stage 26. Similar facts about the ear-vesicl are known from the work of Röhlich and Sidorov.

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* There has always been much difficulty in translating the terms "*ortsgemäss*" and "*herkunft gemäss*" into English. Grafting, however, has long had English words; there is the stock or plan on which the graft is made, and there is the scion (from the Latin root *seco*, to cut) or piece cu off from another plant and grafted. Hence we might use, as A. L. Peck suggests, "stockwise for "*ortsgemäss*" and "scionwise" for "*herkunftsgemäss*". But since there is a confused vulgar us of the word "scion", I prefer "neighbourwise" and "selfwise" and I have adopted them i this book.

One region of the amphibian embryo, however, is much less plastic than the rest during the early part of development. This is the region of the dorsal lip of the blastopore, which has arisen from the grey crescent and will in time form the mesoderm, i.e. notochord, somites, etc. If a piece of this region is grafted into another embryo in the blastula or early gastrula stage, it will "induce" (see p. 104) the neighbouring host tissues to form a secondary embryo, often including nerve tube, brain, eyes, ears, somites, notochord, etc., irrespective of the presumptive fates of those tissues. In other words, it contains within it the influence which determines the fates of regions with which it comes in contact. It is therefore called the *Organiser* (*Organisator* of Spemann). It is easy to demonstrate its fundamental importance in the development of the amphibian egg by separating the first two blastomeres instead of killing one of them, as in Roux's experiments. Their subsequent fate will now depend on whether or not they contain a sufficiency of the region which will afterwards become the organiser region. If both blastomeres contain a portion of it, both will be organised into small but morphologically normal embryos, in accordance with the original experiment of Driesch. If one contains all of it and the other contains none (as will happen if the first cleavage chances to be horizontal), the former will produce a complete embryo and the latter will give up the struggle, as it were, after a few cleavages. These effects were described by Herlitzka as long ago as 1896, but the exact analysis of them awaited the beautiful constriction experiments of Spemann[1] and Ruud & Spemann. A newt's egg constricted into two in the transverse plane will give from the dorsal half a well-formed embryo and from the ventral half a blastula-like ball of yolk-cells developing no further ("*Bauchstück*"). But if it is constricted so as to separate lateral halves, it will give two perfect half-size embryos (see on, p. 228). Schmidt[1] has shown that the same is true for the frog. On the other and, fusion of two amphibian eggs into one is possible; it was accomplished y O. Mangold & Seidel. The organisation centres may fuse and produce a ingle neural axis on a giant embryo.

We now understand better the conditions necessary for the fulfilment of Driesch's pluripotency.

2·12. Morphogenetic stimuli

The essentially new thing in Spemann's conception of the organisation centre or Organiser was that it set the process of *Dependent Differentiation* right at the heart of normal development. Only after this process has taken place, and indeed as the response to induction, does self-differentiation proceed. The distinction between dependent differentiation and *Self-differentiation* had originally been made by Roux[2,5], who said that there were some organs, tissues or regions in embryos which possessed within themselves at a given stage all the factors necessary for the realisation of their normal fates, while there were other organs, issues or regions, which for the realisation of their normal fates required the nterposition of factors external to themselves. It is of interest to quote Roux's own words:

"Self-differentiating structures are those which change themselves as the result of causes lying within themselves. This is apart from necessary external fac which might be called pre-conditions, such as sufficient nourishment, oxy and warmth. It must also be supposed that these external factors have no spe effects, controlling the quality, place, time, and magnitude of the change. T do not affect the time, if the change happens no earlier than normally, even tho they may be present earlier than normally; nor the place, if they are presen places where the change is not going on. They do not affect the intensity, i spite of changes in them the magnitude of the embryological change is unalte nor the quality, if the change is a morphological one....

Structures undergoing dependent differentiation are those which change response to causes lying entirely or to a substantial degree outside themsel The nature of the change, and its time, place, and intensity, may be determi from outside the structure. If all of these depend upon external conditions, differentiation will resemble the formation of a statue from a marble block, such extreme dependent differentiation we may call passive differentiation..

A given differentiation may be dependent in respect to one or other of th factors, if not to all; this can be called mixed differentiation. These numer possibilities make the complete analysis of all the causes of a morphologi change exceedingly difficult and complicated.”

Thus the distinction was fully made by Roux, but it appears that the kind factors which he pictured as acting upon embryonic rudiments in depende differentiation were mainly or entirely the physiological stresses and strains the later period now known as that of *Functional Differentiation*. Thus the sha of the blood-vessels would be affected by the hydrodynamic forces of the circ lating blood, the bones would develop in accordance with certain mechanic stresses, etc. Roux did not clearly envisage the stimuli as chemical.

The concept of morphogenetic stimuli had, however, two other quite differe sources. As early as 1858 Virchow developed it in relation to the production specific types of tumour in man. This subject was further elaborated by Billro in 1890, who attributed these effects to definite chemical substances elaborate by the stimulating organism, insect, worm or bacillus. We shall shortly retur to the interesting and undeservedly neglected subject of gall-formation. B in the meantime the botanists had been deeply engaged in the study of tropis and taxis, and the word *Induction* was used for the first time in this connectio by Pfeffer in 1881 in his consideration of the “Induktion spezifischer Gestaltung by factors external to the plant, such as light or gravity.

The existence of chemical stimuli in embryonic development was fir thoroughly brought forward, it seems, in the articles contributed to the *Biologisch Centralblatt* in 1894 and 1895 by C. Herbst[2], as the result of his experiments wit sea-urchin embryos cultivated in artificial salt solutions from which single ion were missing (see *CE*, Section 13·4). He set out to consider the significance o stimulus-response physiology for ontogenesis and classified all the possible kind of stimuli, starting with external factors such as light, gravity, temperature, etc The line of thought followed began with the tropisms of free-swimming organism and the taxis of plant tendrils, etc., going on to describe the accumulation of cell at the surface of the arthropod embryo to form the blastoderm as an “oxygeno-taxis”, and the penetration of vitellophages into the yolk in other forms as chemotaxis. From this it was not far to think of other chemical substances which might have as their typical response, not movement, but morphological change. A botanical example, the production of swellings by vine tendrils on contact with a solid surface, lay close to hand, though here the stimulus was apparently mechanical, not chemical.

The actual examples which Herbst could give of chemical morphogenetic stimuli were in fact rather few; apart from gall-formation, to which he devoted a good deal of space, there was the origin of the antheridia stalks in *Saprolegnia* (secretion of a diffusible substance from the oogonia leading to the formation of the male antheridium-bearing branches), and the exogastrulation produced by lithium salts in echinoderm embryos, though this last could hardly be regarded as a normal internally produced stimulus. "The exact investigation", he wrote, "of the numberless cases of induction of specific morphological forms by internal factors will form a fundamental aim of future biological researches." But already Herbst was able to give a theoretical outline to problems which have only become acute in our own time. Thus from the study of galls, he drew the conclusion that one stimulus might have quite different responses if it acted on different tissues, or that one stimulus might give rise simultaneously to more than one type of response. On the other hand, some tissues might give a single type of response to a number of chemically different stimuli. Again, the response to stimulus might depend very largely on the stage of development reached by the reacting tissue, which in early stages might have no reactivity, and in still later stages might lose it after gaining it. This was illustrated by the fact that galls are much less commonly produced on fully differentiated tissue than on buds, young leaves, etc. All these suggestions of Herbst will be better appreciated when some of the facts of modern experimental morphology have been described in what follows (cf. p. 261), but they constitute a brilliant intuition of the ideas which studies on hormone specificity have now compelled us to adopt.

Morphogenetic stimuli were at this time definitely "in the air", for in 1893 C. Emery, a student of bacterial tumours, suggested that "some morphogenetic stimulating substances probably exist in the egg, others arise during development at different times and in different organs one after the other". At the turn of the century, however, the stand taken by Herbst was fully justified by the analysis of the development of the amphibian eye undertaken by Spemann[2] and W. H. Lewis[1]. It was found that if the eye-cup were in any way prevented, as by removal or injury, from coming into contact with the overlying head ectoderm, no lens would be formed. Lewis[2] also showed that if the eye-cup was allowed to act on ectoderm from any part of the body, or even on ectoderm from another species of frog, a lens would be perfectly formed. Separated into several parts, the eye-cup pieces still possessed the power of lens induction. In 1901 Herbst's articles, very much rewritten, were published as a book, *Formative Reize in der tierischen Ontogenese*. He had unwittingly postponed the appearance of his book just long enough to enable him to mention these new results of Spemann's.

"Gerade als mein Manuskript so weit fertig war, dass es zur Post gehen könnte, erhielt ich durch die Güte des Herrn Kollegen Spemann den Bericht einer Sitzung der physikalisch-medizinischen Gesellschaft in Würzburg Anfang Mai 1901, aus dem hervorgeht, dass Spemann die gleiche Ansicht ich von der ontogenetischen Entstehung der Linse des Wirbeltierauges hat. gelang ihm nämlich, folgende beide, für unsere Frage wichtige, Punkte durch Experiment sicherzustellen: '(1) Wenn der Augenbecher die Haut nicht errei so bildet sich keine Linse....(2) Sowie der zurückgebliebene Augenbecher Epidermis berührt, beginnt an der Berührungstelle die Wucherung der Linse

The early history of this subject certainly seems to suggest that the right id came some time before the proofs of their rightness.

One passage from W. H. Lewis[1], written in 1903, demands quotation, in light of modern knowledge:

> "It is not unlikely that a new biological chemistry must be developed, reactions being those between living tissues or between a living tissue and t product of a living tissue. In the example of the lens reaction it seems evide that living ectodermal cells must be acted upon, but it is not so evident that t living optic vesicle cells are necessary, as it may be possible to extract from the substances which will give the lens reaction. By means of such a biologic chemistry some of the properties of the various tissues and organs may discovered and perhaps a deeper insight into the problems of developme obtained. The development of a biological chemistry is to be considered as b one of the phases of correlative embryology, and in considering the correlatio in development, the mechanical factors must not be neglected."

It would be interesting to know whether these pioneer embryological investigato were at all influenced in their interpretations by the pioneer work which wa going on at the same time on the hormones of the adult mammalian body. Th first of these studied was the active principle of the adrenal medulla, by Oliver Schafer in 1894. Its formula was already worked out by Takamine in 1901, an in the following year Bayliss & Starling made the discovery of secretin, afte which endocrinology made rapid strides forward to its present position.

2·13. A digression on plant galls

In Herbst's thought we have seen how the earlier investigators were led t the conception of morphogenetic stimuli, and we do indeed find in certai interactions between animals and plants phenomena which must be ascribed t the action of morphogenetic hormones. The whole subject of the plant galls, as for example, described in the monographs of Küster and of Ross & Hedicke, i the best illustration of this, although up to the present the relation between thes forms of "dependent differentiation" and what we now know of organise phenomena in animals has not attracted much research. A fascinating field lies open here for biochemical exploration and experimental analysis.

As is generally known, galls arise from the abnormal development of meristematic tissues of plants as the result of a stimulus usually caused by an insect parasite. In many cases the exciting chemical substance seems to be contained in the juice injected into the plant by the ovipositing insect at the same time as the egg; in other cases, the substance is apparently produced by the larva as soon as it hatches. But the important point for the present argument is that, although the gall is built up from the tissues of the plant, its morphological form depends upon the nature of the exciting animal. Thus the same plant attacked by different insects will produce very different galls. It is said that in some cases the morphology of the galls may be an easier method of distinguishing between closely related species of insects than the morphological characters of the adults themselves. The usual instance given of this is the American willow, *Salix humilis*, which is attacked by ten different, though closely related, species of gall-gnats (Walsh). Fig. 39 shows one kind of gall produced by such an insect (*Rhabdophaga rosaria*) on the willow. Again, different parts of the same plant may be attacked by the same insect with the same result. The currant galls of *Spathegaster* (= *Neuroterus*) *baccarum* occur both on the leaves and the flower stalks of the oak, so that their morphology cannot be a function of the part of the plant that produces them. Finally, the same gall may be produced by the same insect on several species of plant, for example, the sawfly *Micronematus gallicola* produces its brilliant red galls on four separate species of willow, *S. fragilis*, *alba*, *caprea* and *cinerea*. The gall of another hymenopteran, the gall-wasp *Rhodites rosae*, on the rose tree is illustrated in Fig. 40. Few workers have studied the histology of the gall throughout its development; the nicest pictures I have seen are those of B. W. Wells.

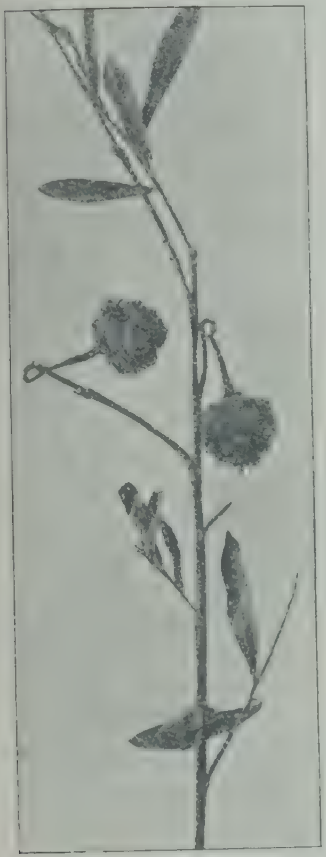

Fig. 39. Gall produced by the gnat *Rhabdophaga rosaria* on the willow.

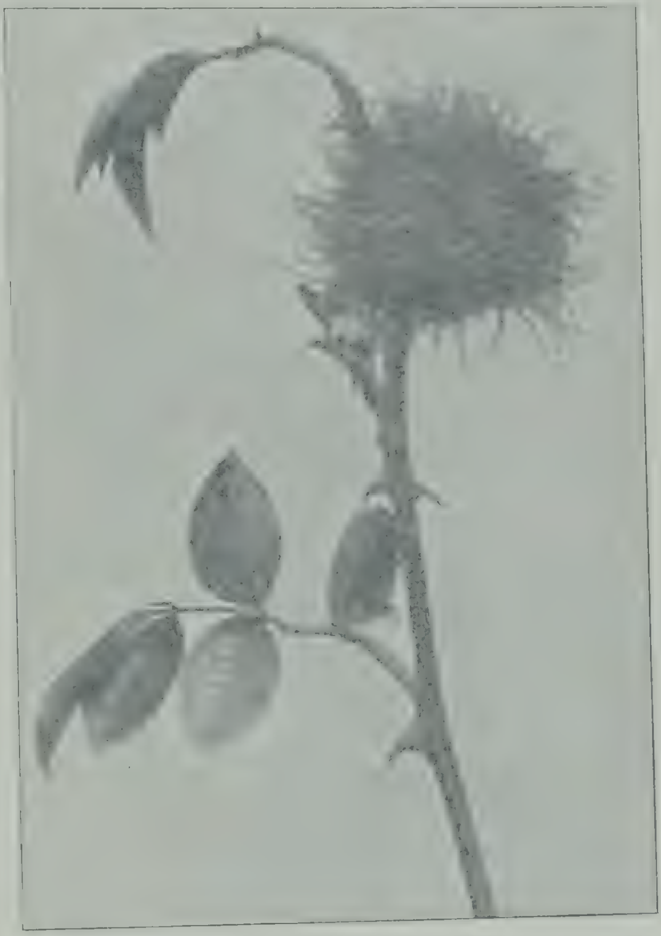

Fig. 40. Gall produced by the wasp *Rhodites rosae* on the rose tree.

All this indicates in the strongest possible manner that in the course of evolution the pursuit of the parasitic habit has involved the preparation of a great number of chemical substances by insects (and some other animals, such as nemato also) which have a specific morphogenetic effect upon the tissues of the plant.* But as yet experiments directed towards proving the truth of this have not been very many, and their success has been but moderate. The famous were those of Beijerinck[1], who as far back as 1888 killed the egg sawflies by puncturing with a hot needle immediately after their inoculation the plant by the insect. In spite of this the galls developed normally. In the of the Cynipid galls, on the other hand, it is certain that the irritating substa is first produced when the larva hatches from the egg, and begins to feed u the plant. Beijerinck called these substances "growth-enzymes", and in a la paper[2] (1897) applied the idea to the development of form in general, which thought was determined in the embryo by the diffusion of stimulatory substanc

It appears that no investigator has yet succeeded in preparing a cell-free extr of an insect or its egg by any chemical method which will, when injected into plant, give rise to the characteristic gall of the species. It is, however, equa true, if we may judge by the review of Küster,† that no investigator succeeded in causing gall-formation by the application of unspecific stim such as the implantation of oils, acids, cantharidin, yeast, proteins, etc. The mo probable conclusion is that no one has yet found the correct extraction metho and indeed the literature shows well enough that no very high level of chemic technique has as yet been applied to the problem. Significant, however, is t finding of Laboulbène that the implantation of small pieces of dead larva successfully produced galls (cf. the persistence of organiser action after the deat of the cells of the organiser region), although this worker made the further clai that the injection of water in which the larvae had been washed would als provoke the characteristic gall. The technical difficulties here, it must be remembered, are rather great, for the reactive layer is presumably the cambium, an as this is supposed to be but one cell thick, the problem of directing a sufficientl intense stimulus to the right place is not easy to solve. It may be recalled th the percentage of positive results in chemical experiments with animal evocato is generally not high, for very similar reasons.

Work which has a direct bearing on this was that of K. M. Smith, who, in ord to study the nature of the damage produced in apple leaves by the activities capsid bugs, implanted the salivary glands of these insects beneath the surfa of the leaf, and was able to produce in this way all the pathological phenome usually caused by the presence of the insects themselves. Although these bu (such as *Plesiocoris rugicollis* and *Lygus pabulinus*) do not form what are, proper speaking, galls, the damage they do is somewhat parallel. The homopter *Asterolecanium variolosum*, however, does form galls, and Parr was successf in reproducing them by injecting glycerin extracts of the salivary glands. Th various organs of gall-wasps were investigated by Rössig, who thought he could trace the site of formation of the gall-forming substance to the Malpighian vessels, and this was confirmed later on by Triggerson for the Cynipid which produces the oak hedgehog gall, *Dryophanta erinacei*. Lastly, the structures produced in the larch by the aphids of the *Chermes* group have been proved by Burdon to be due to the injection of a chemical substance, which in this case is relatively unspecific, since all the species produce the same effect.

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* The effects of some parasitic worms on animals might be classified as galls, e.g. the hydat cysts produced in the human liver by the flatworm *Echinococcus granulosus*, or the sarcomas ar hepatomas caused by the cysticercus larvae of *Taenia crassicollis*. At this point we reach t borderline between parasitism and true malignant tumours, but so far extractable carcinoge have been sought for in these organisms in vain.

† *Gallen*, pp. 279 ff.

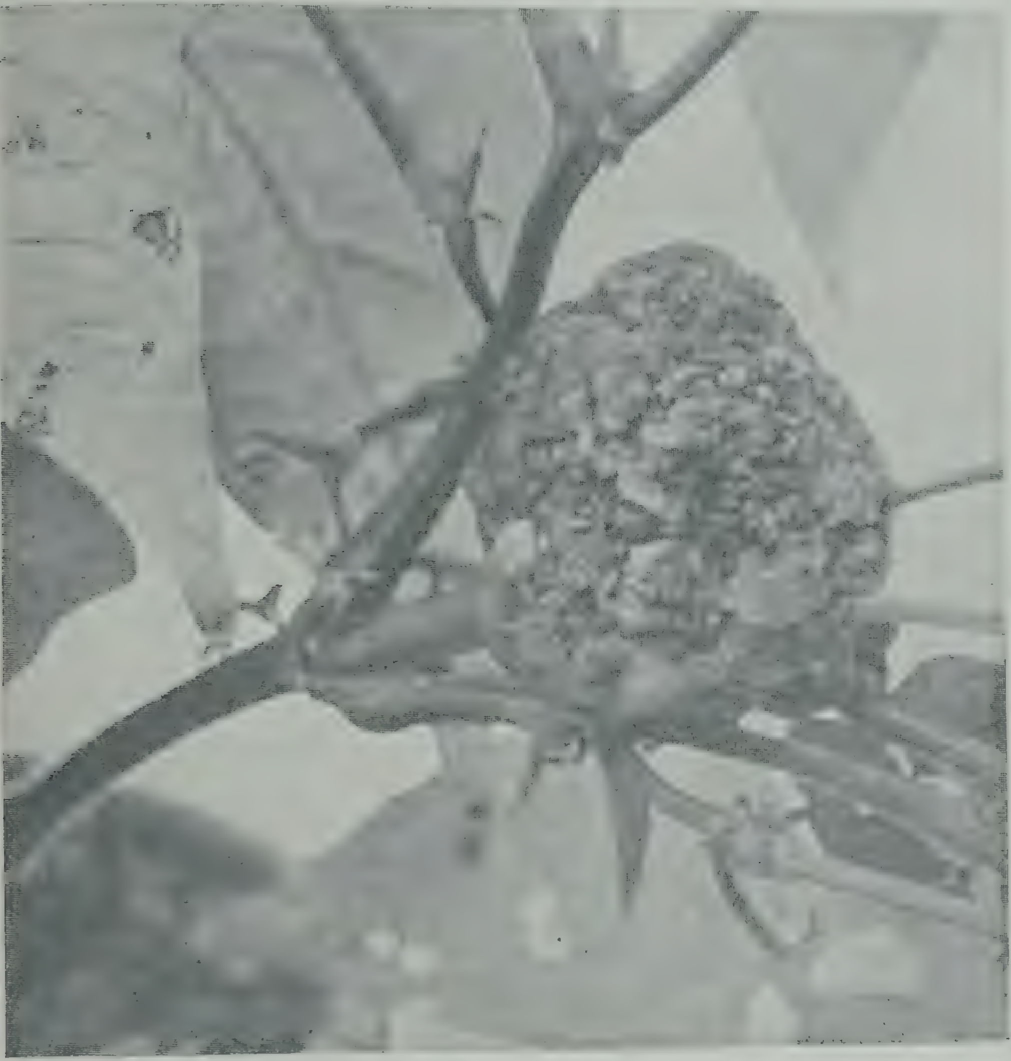

Fig. 41. Gall produced in five months on a stem of *Phaseolus* by applications of hetero-auxin.

It is now known that the plant-growth hormone auxin, which we shall have occasion to mention again from time to time in the organiser story (pp. 212 and 266), has a good deal to do with galls and plant tumours. In 1912 Molliard produced tuberisation of the roots of pea plants by subjecting them to the action of the products of *Rhizobium radicola* in cell-free extract. Thimann[2] has now proved that the root nodules of leguminous plants, of such fundamental importance to man in the history of agriculture, are almost certainly due to the auxin produced by the invading nitrogen-fixing bacteria. The crown-gall organism, *Pseudomonas tumefaciens*, also produces auxin (Brown & Gardner; Solacolu & Constantinesco). These authors succeeded in obtaining typical crown-galls by applying a hetero-auxin in lanolin paste to a *Phaseolus* stem for five months (see Fig. 41). Sim treatment with hetero-auxin, but at lower concentrations and for a shorter ti allowed la Rue[1] to simulate perfectly the intumescences which arise under c ditions of high humidity on the leaves of the poplar, *P. grandidentata*. In a la paper[2] the same author showed that the faeces of larval leafminers contain au and that this produces the proliferations of the host plant.

The whole subject of plant tumours has been reviewed by Levine; Rose, a Gheorghiu. While auxin and related substances doubtless play a great part the causation of galls and tumours, the high specificity of many insect spec in the morphology of their galls suggests that many other specific stimulat substances must be involved.

2·14. The hierarchy of organisers in animal development

By 1909 the concept of dependent differentiation by the action of chemi morphogenetic stimuli was well known, and Jenkinson, writing in that ye could say:

> “Should the results of these extremely interesting investigations be confirme the development of some parts, at least, of the vertebrate eye would be process of ‘dependent differentiation’, dependent, that is, on causes outside themselve on stimuli exerted by other parts....How far such an action of the parts upo one another is of general occurrence as a factor in differentiation, future researc alone will show. It seems probable, however, that it will be found to be limite to the first moments in the formation of organs, for we know, from other evidenc that as development proceeds, systems which were equipotential becom inequipotential, parts gain independence and the power of self-differentiatio and the value of the correlations between them diminishes.”

Thus the morphogenetic stimuli were being linked up with the first process determination, whereby the fates of the parts of the embryo are fixed, rather tha with the further histogenetic elaboration of the parts.

After the discovery in 1924 of the inductor which stimulates the dors ectoderm of the amphibian gastrula to form the neural tube, Spemann[12] introduce the term *Primary organiser* for it, since it is the first to work in the developmen process. Seats of processes which occur later on, such as the induction of th lens from the ectoderm by the eye-cup, he termed *Secondary organisers* (c Second-grade organisers), for if no neural tube were formed there would neve be any eye-cup. Third-grade organisers also exist (see pp. 301 ff.).

Development, then, consists of a progressive restriction of potencies by determination of parts to pursue fixed fates.* There is a growing conviction that thi tate of affairs can best be pictured in the manner of a series of equilibrium states. The whole process could then be imagined in the likeness of a series of cones (Fig. .2). At the top of the uppermost cone there is a ball in a position of extremely unstable quilibrium. It will tend to fall along the side of the cone and will reach a point t some one of the 360 degrees of the cone's circumference. Here it will again find itself in a position of unstable equilibrium, only now with respect to a second stage of determination, and will again be pushed in one direction or another, again to occupy a passing equilibrium, and so on until the final stage of absolute stability is reached, i.e. the plan of the adult body. In biological terms a piece of ectoderm will be determined first of all by the primary organiser to form head ectoderm, next it will be determined by the secondary organiser of the eye-cup to form the lens of the eye, and so on. The effect of the primary and secondary organisers in the normal course of events is to give the slight pushes necessary to send the balls down the cones into specific positions of greater stability as time goes on.* A grafted organiser will give a push of this kind to a given all (plastic region) in a direction ontrary to that which it would ormally have taken, but for this it s necessary that the ball should be high up the levels of instability, for, if it as already been severely determined, the grafted organiser can have no effect upon it. Conversely, an organiser of the second or third grade may have no ffect upon a ball (plastic region) at a higher level of instability than that at which it normally works. The undetermined material is then said to be not yet "competent" to receive its action.

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* Qualms about the determination concept were voiced by Harrison[12] in 1933. His main difficultie were (1) double assurance of eye and operculum, (2) *bedeutungsfremde Selbstdifferenzierun* and (3) the fact that the ascidian, apparently so determined as an egg, is so capable of regeneratio as an adult. The first of these is now a very doubtful question (pp. 293, 308); the second has definitely been proved not to happen in neutral medium (p. 157), and the third presents less difficult if we admit, with Conklin[3], that determination may be reversible. Zhinkin[3] has actually followe the appearance of regenerative and regulative power (*Recuperation*, see p. 442) during ascidia metamorphosis. In amphibian regeneration, as we shall see (p. 435), the generally accepte pluripotency of the blastema rests on insecure foundations. I ally myself in this book with thos embryologists who are not prepared to give up the word “Determination”, always subject to th conditions of employment given in the glossary.

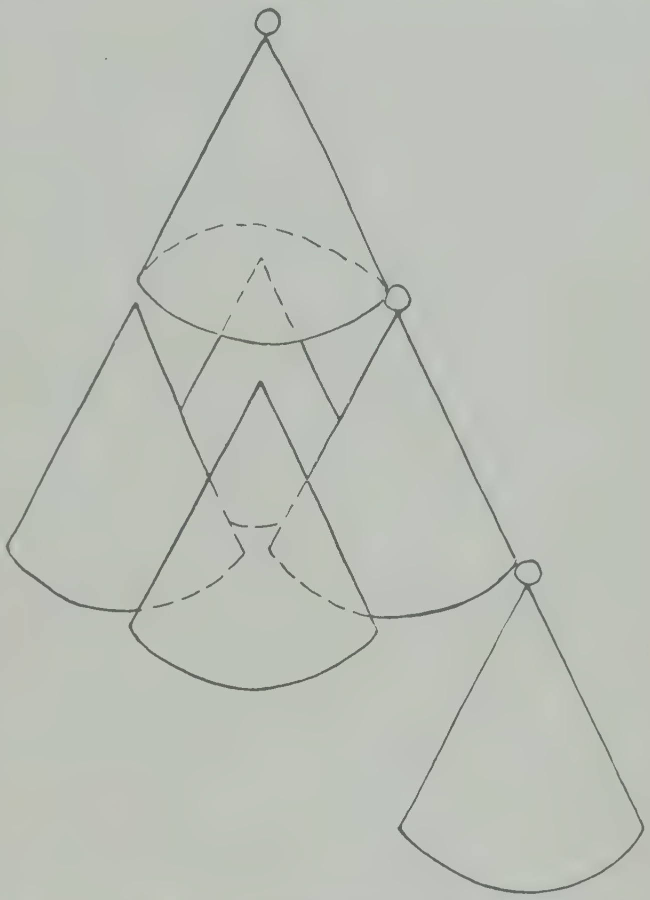

Fig. 42. Action of organisers of successive grades visualised as involving a series of equilibrium states.

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* Roux's idea of a struggle of the parts has been revived in a new form by Raven[5] and others, who picture a "struggle" or competition between the various tendencies in a developing piece of tissue. Raven seems to think that tendencies are more than competences, since they manifest themselves in self-differentiation, but if we adopt the view that there is no self-differentiation without prior induction, competence and induction are the only two concepts required. How exactly n inductor selects and puts into effect its chosen competence is a question which we shall not be ble to answer until we know what competence means from a physico-chemical point of iew.

2·15. Competence and unstable equilibrium

In all these considerations, we must bear in mind that the state of the reac
tissue is just as important as the nature of the stimulus provided. Determina
and hence, Induction, can only occur if the ectoderm, for example, is in a reac
state; if the ectoderm is too young during the period of application of the stimu
no effect will be produced; while if the ectoderm has already been determi
to be something else, no inducing stimulus can alter or reverse that determinat
This state of reactivity* is known as *Competence*.† The roof of the blastocoel
a blastula is not yet competent to react to the stimulus of the primary organi
On the other hand, the cells of the neural folds were once competent, but
so no longer. In isolated gastrula ectoderm studied by Waddington ([12], p. 81)
Holtfreter[18] (see p. 281), competence disappears after a certain time without
determination having taken place. The ectoderm eventually ceases to be abl
respond to an organiser, even if one is implanted.

It is worth while to follow the arguments with which Waddington[1] introdu
the concept of "Competence". Much work had been done before that ti
(1932) by the American school, and has been continued since, in order to stu
self-differentiation, on the transplantation of pieces of chick blastoderm to
chorio-allantoic membrane of the egg. Such a site was then regarded as a qu
indifferent one, which could not influence differentiation. Hoadley's series
papers[1] had made it clear that various regions of the blastoderm possess t
capacity for self-differentiation from very early stages of development onwa
and that this capacity increases with age. These results were formulated
F. R. Lillie[9] in terms of a process of "differential dichotomy" or "embryo
segregation", in which the capacity of the embryonic cells for self-differentiati
became itself differentiated.‡

According to Hoadley[1], an early embryonic cell should only be capable
self-differentiating into a rather generalised cell-type of low organisation. As t
process of differential dichotomy goes on, however, the descendants of such
cell acquire more specialised potentialities which have been carved out of t
original general potentiality by a process of segregation. Thus, if a piece of tiss
was isolated from an early blastoderm the segregation process would cease, a
it would differentiate only into epidermis and gut; its weak capacity for sel
differentiation would carry it only a short distance along a general path. If

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* States of reactivity are important wherever stimulus-response relations exist, as in all neural a
humoral activity. Thus in the phenomenon of prepotence, studied by Sherrington, a nervo
impulse coming from a nociceptive afferent arc has priority over other reflex impulses for the u
of the final common path. The efferent arc could thus perhaps be said to have numerous co
petences, only one of which can be realised at once. Pharmacology, moreover, abounds
instances where reactivity can be experimentally altered.

† This word supersedes the older "Potence", "Potency" or "Potentiality" discussed elsewhe
(p. 100; p. 436 and glossary).

‡ The word segregation was derived from Ray Lankester's discussion of the "gastraea" theor
But he meant by it not a segregation of competences but a segregation of the "physiologic
molecules" that are going to form "deric and enteric" (i.e. ectodermal and endodermal) materi
We can hardly call this a concept of determination, because the possibility of alterations of pr
sumptive fate was not then known (1877), but that is its nearest kin.

similar isolation was made at a later stage, an eye would be obtained; the stronger capacity of the daughter cells carrying them a longer distance along a more special path.

Now the idea of segregation contained two notions; first, that the process gives rise to parts with new potentialities, as Hoadley concluded, and secondly, that it nvolves a restriction of potentiality from a general to a particular. F. R. Lillie[9] defined it as the "process of origin of parts of the embryo possessing irreversible prospective significance as tested by self-differentiation". But unfortunately self-differentiation techniques do not in fact test irreversible prospective significance. In the newt the ectoderm has the capacity for self-differentiation to epidermis at a stage before it is restricted to this fate. True irreversible prospective significance can only be tested by transplantation experiments in which the tissue s given the chance to prove itself immune to the influences of the host's inductors. But if segregation is limited to being the process of origin of parts possessing rreversible prospective significance as tested by transplantation experiments, it becomes identical with determination, and it loses the first notion mentioned above, namely, that it is responsible for the origin of parts with new potentialities.

New potentialities, however, do in fact arise. In the differentiation of neural plate from ectoderm in the newt, for example, the tissue cannot at first react to the stimulus, it then enters on a period when it can do so, and if the stimulus is applied, the differentiation takes place; if not, the competence may be lost. Whenever competence for specific differentiations arises, new potentialities arise. We shall see later on (p. 155) what can be said about competence and its rise and fall.* But in the meantime, it is clear that since several competences may exist side by side in the same cell region, there is nothing to be gained by referring to their origin as due to dichotomy.†

Lillie's famous paper[9] revealed indeed a very Laocoon striving to dominate the oils of terminology. He introduced the term "specific potency" to mean reversible prospective significance (i.e. the determined state), thus genuinely onfusing the concepts of power and possibility. A piece of an embryo has the *ossibility* of a certain fate before determination, and the *power* to pursue it fterwards. The use of "potency" for both these meanings was indefensible. Segregation" also confused two things, the time at which a certain competence rises and the time at which the determination of a competent tissue is effected. Other expressions introduced by Lillie, such as "open and closed term" for ndetermined and determined stages; "isotropic" for pluripotent (though sotropic has a definite meaning in physical chemistry, see p. 662, inapplicable to he egg); and "self-origination" (a word which would have pleased Athanasius)

The idea of competence covered two concepts previously in use by the German school—*eaktionsfähig* and *labil determiniert*, "capable of reaction to a given stimulus" and "possessing abile determination". Labile determination meant that a tissue was capable of differentiating to ome extent in a given direction even without further external stimulus. If the external stimulus as given, the normal fate could then be said to be doubly assured. The existence of labile etermination and double assurance is, however, very doubtful (see below, pp. 293 and 308).

There is no reason why sixty different competences should not exist side by side. The oak tree ill form some sixty different sorts of galls. We know as yet practically nothing about the origin of ompetences.

for the alleged appearance of a lens without the stimulus normally coming the eye-cup (see p. 293); all these are equally unsatisfactory and cannot long retained. Lillie was, however, the first to say in so many words that embry potencies are a function of the specific proteins in the regions where they oc a point to which we shall often return (pp. 203 and 670).

Now to be "reaktionsfähig" or competent with respect to several fates be in a condition of unstable equilibrium. Whatever provides the stimulus the realisation of one of the fates which the competences represent will usu have the effect of suppressing the remaining competences or degrees of freed and so of inaugurating a condition of more stable equilibrium.*

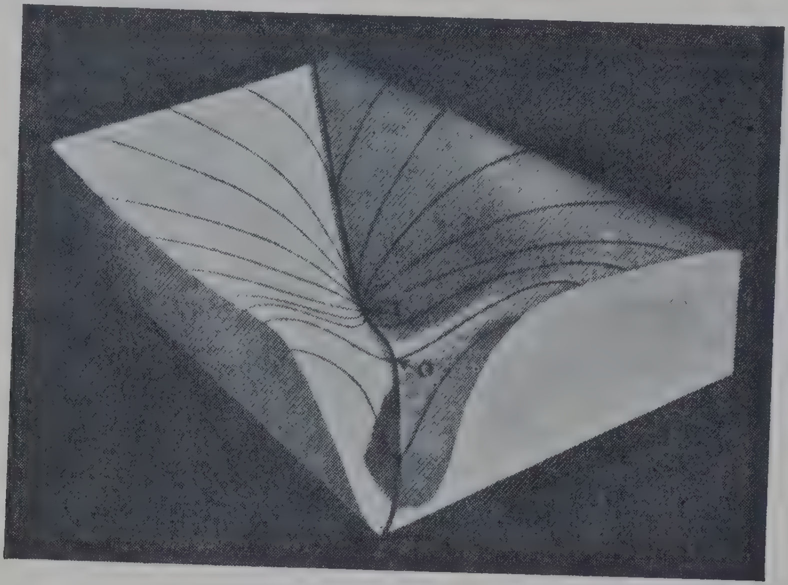

Fig. 43. Model showing the interpretation of integral curves for the Ross malaria equation as lines of steepest descent on a topographic surface (see text).

The concept of unstable equilibria in early development seems to offer oppo tunities for advance in the mathematisation of embryology. Lotka, who h devoted much study to the use of equilibrium concepts in biology, gives a l of the various types of equilibria which may be met with. If only two variabl are in question, the integral curves may be plotted on rectangular co-ordinate Families of curves will be obtained passing through the equilibrium points, whic will appear as singular points. As an example, Lotka figures the topograph chart of the well-known Ross malaria equations, which describe the course events in the spread of malaria in a human population by the bites of certai breeds of mosquitos infected with the malaria parasite. In this chart there a two singular points, one at the origin, *O*, unstable, the other at *T*, stable. Th chart, Lotka points out, obviously suggests stream-lines and a three-dimension model. Such a model is shown in Fig. 43. The feature of interest is that a singula point like *O* is represented by a col or saddle-point in the landscape, so that th ttainment of stable equilibrium would be represented by the fall of a point rom O to the pit T. The model serves, as Lotka says, to bring out an important act, namely, that there are necessarily certain regularities in the occurrence of he various types of equilibria. Thus two pits of the character of the point T annot occur without some other type of singular point between them, just as it s not possible for two mountains to rise from a landscape without some kind of alley between them. Now the series of equilibria met with in embryonic letermination might be represented by a series of horizontal ledges descending from a point analogous to O, separated perpendicularly from each other by ridges or cols. Such a model, purely qualitative and intuitional, is represented in Fig. 44.

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* "Competence is a name for the actual state of the tissue at, and before, the time when th instability is resolved and one or other path of development entered upon. Lillie's segregation wa a segregation of potencies, which are not in any way descriptions of the state of the tissue at th time of the segregation, but merely formal attributes derived from a consideration of its futur behaviour" (Waddington[25], modified).

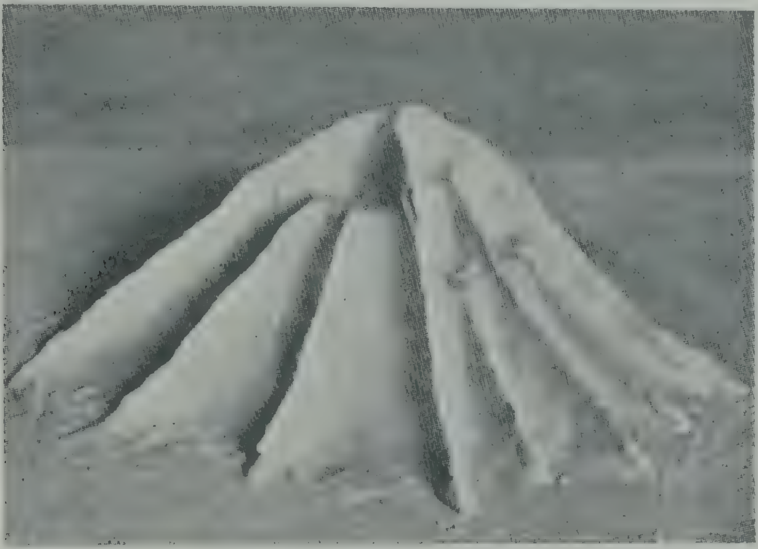

Fig. 44. Qualitative three-dimensional model of embryonic determination, illustrating the transition from unstable to stable equilibria.

he state of "harmonious equipotentiality" would then correspond to the summit oint, where the instability is maximal, and a point could descend to the successive vels of instability not in one direction only, but in several, according to its osition and other relations to the organiser region.* This is to say, its proective potency is not limited to its prospective significance. The true mosaic

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Stability landscapes are being found useful by various writers on these subjects. Thus addington (*Introduction to Modern Genetics*, pp. 182, 186, 190) symbolises the genotype in evelopment as a system of contours enclosing a valley along the bottom of which run the mbryonic processes forming the phenotype. Out of the main valleys branch subsidiary valleys; lternatives which, under certain circumstances (changes in the genotype), the embryonic processes ould have to pursue, leading of course to a changed phenotype. This analogy is not entirely appy, since side valleys normally branch *into*, not *out of*, the main ones. A river delta would be nore appropriate. But the principal distinction between this symbol and the one here proposed s that for a given individual organism not all Waddington's side valleys are live issues, since the enotype only contains a limited number of mutant genes. Some of them are, for we know that y the action of external conditions (temperature, radiant energy, etc.) genic effects can be imitated a given organism (the "phenocopies", see p. 392). In the mountain model of embryonic etermination, however, all the alternatives are live issues, depending on the primary rganiser and the chain of inductors. S. Wright[3] has also used the topographic surface or robability landscape for his interesting arguments about genes and evolution; unfortunately e makes the highest hill-tops the points of maximum stability, which reverses the sense of e symbol.

egg, such as that of the polychaete worm *Sabellaria* (Novikov[1]), would n occupy any of the higher ledges.*

Inorganic solutions or vapours may exist in the condition of supersatura and they may then be brought to crystallise or to condense by the introduc of a suitable nucleus, the dimensions of which may be minute relative to system as a whole. This state of affairs is called a metastable state. Ther dynamically it is defined by the fact that its potential is a minimum, but no absolute minimum. The term metastable could justifiably be applied to plateau-like states which occur in the above model in embryonic determinat The appropriate organiser or inductor would then correspond to the nuc which ends the state of supersaturation in an inorganic system. It is reminisc of Bertalanffy's[1] phrase,† that the egg is "charged with morphological form" has a "morphological charge". Just as inorganic metastable systems are st in the absence of their nucleus, so, in the absence of the organiser or induc normal development and differentiation will not occur, as is beautifully seen the exo-gastrulation experiments of Holtfreter[8] (see p. 159).

This subject may also be regarded from another angle, i.e. the appearance a accumulation of the chemical substance which is the inductor in any given ca The morphogenetic hormone or inductor will be the product of a chemi reaction, appearing from a precursor substance, or being liberated from inact combination, and possibly open to destruction by other metabolic reactions that its effective concentration at any given time may depend on several facto Fig. 45 shows the situation in generalised form. The horizontal axis is time (duri embryonic development); the vertical axis is the concentration of the inducto The period of competence of a given tissue for a given fate will then be a zo between the times c_1 and c_2. The inductor may be formed or liberated at differe rates; if its critical threshold concentration (the horizontal line i, i) is attain before the beginning of competence, as by curve A, then the determinati process (d_1, d_2) will begin as soon as competence begins. As an example, t treatment of amphibian larvae with thyroxin from an early stage onwards mig be mentioned; metamorphosis will begin as soon as competence to react to t hormone begins (cf. p. 448). In most cases of early embryonic determinatio however, the competence is present before the necessary amount of induct (cf. p. 154), so that curve B represents the state of affairs. Here the determinati process will start as soon as the threshold level is attained. Further increases the amount of the inductor will not add to the morphological effect. If t cessary amount of inductor fails to reach the tissue before the end of the npetent period, as in curve *C*, no induction will ever take place. Amphibian -gastrulation would be a case in point (cf. p. 159), or isolated ageing ectoderm . p. 281). This right-hand region of the graph also represents the state of affairs, we shall see, in adult tissues, where inductors are present, but no competence . p. 174). We need not regard the period of competence as absolutely fixed; may be shifted to the right or abolished altogether. Similarly the threshold ncentration might be raised, as indicated by the arrow on the right-hand side the graph. These changes would lead to the suppression of inductions. As we ll see later, many cases of such suppression are known, both in nature (p. 222), erimentally (p. 224) and by the action of mutant genes (p. 368), but we cannot say which of these mechanisms is responsible. There is some evidence,

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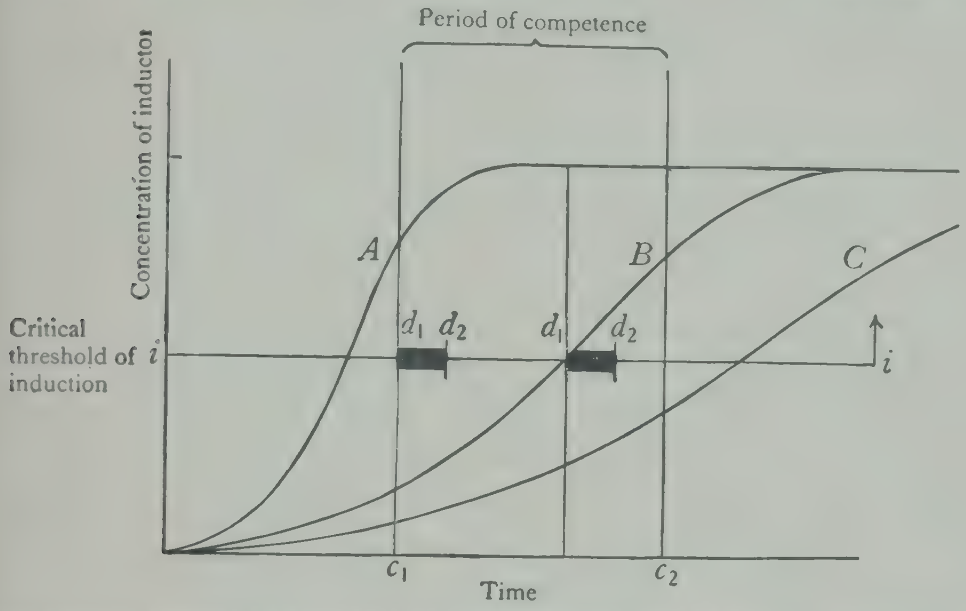
* It would, of course, do so in pre-fertilisation stages. The equilibria here envisaged, whi become ever more stable as development proceeds, approximate to previous uses of the conce in biology. Thus S. J. Holmes and Przibram (*Anorganische Grenzgebiete d. Biol.* pp. 64 ff.) ha spoken of the completed organic form of the animal as being an equilibrium which is disturbed e.g., the removal of a limb. There ensues a struggle between growth and surface tension or a m action effect, ending in an equilibrium which *is* the normal form once more. For what is actua known about regeneration in vertebrates, see pp. 430 ff.

† Bertalanffy (*Kritische Theorie d. Formbildung*, p. 223): "So, wie ein Akkumulator mit Ele trizität geladen ist, so ist gewissermassen das entwicklungsfähige Ei mit 'Form' geladen, die in Wirklichkeit umzusetzen trachtet." See also his article *Tatsachen u. Theorien d. Formbildung Weg zum Lebensproblem* in *Erkenntnis*.



45. Generalised scheme of action of an inductor visualised as itself a metabolic product.

ved from studies of avian feather development (see p. 303), that the level of threshold may be connected with the growth-rate. The faster the part is wing the larger the amount of an inductor is required to affect it. The period ompetence might also be shifted to the left; in this case the determination and erentiation of organs would occur before the normal time. Phenomena such rothetely in insects, where organs characteristic of a later stage may appear he larva, may perhaps be explained in this way (cf. p. 239).

he above formulation has the advantage that it links up the study of embryonic anisers with the ideas of Goldschmidt on the production of morphogenetic and er hormones in the embryo at rates dependent on the particular genetic equip-nt of the individual. Animals vary not only as to the presence or absence of a nber of organs, but also in the extent to which any one of these organs is formed. animal might have all its organs complete except one, but that one might be rely absent. On the other hand, it might have them all, but in incomplete n. Such organisms have been called mosaics* and intermediates respectively.

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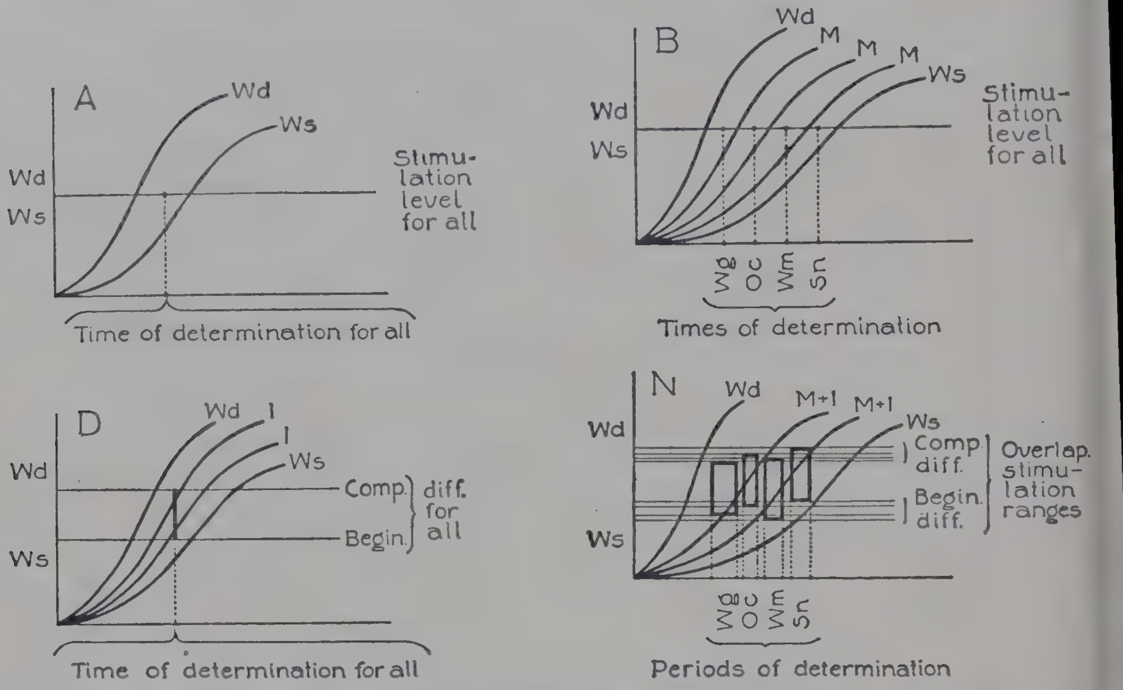
* Not to be confused with the two other uses of the term. See Glossary.

Shull's work on aphids (*Macrosiphum solanifolii*) led him to use a sch similar to that just described, which is worth examination because it shows h numerous are the effects which may be produced by one substance if it indu several morphogenetic processes (as for example thyroxin does in amphib metamorphosis). In Shull's aphids the winged type differs from the wingless having (*a*) wings, (*b*) three ocelli on the head, (*c*) wing muscles in the thorax, a (*d*) 15–18 sensoria on the third segment of the antennae instead of 4–6. Th characters will be referred to as Wg, Oc, Wm, and Sn in what follows. Now line of demarcation between winged and wingless could be a definite criti


A
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Overlap.
stimu-
lation
ranges
Wg
Oc
Wm
Sn
Periods of determination

Fig. 46. Schemes of inductor action explaining the occurrence of mosaic and intermediate individuals in aphids.

threshold of inductor concentration, the same for all the structures, and all th structures might have the same moment of determination. In this, the simple case (Fig. 46 A), a rate of formation of the inductor represented by any curve the left of the point in question would result in the production of winged ind viduals (Wd), while a rate of inductor-formation represented by any curve to th right of the point in question would result in the production of wingless in dividuals (Ws). There would be no intermediates and no mosaics.

Intermediates would occur, however, if subsequent differentiation depende upon the concentration of inductor which had been formed at the moment determination. This is shown in Fig. 46 D. The effective concentration of induct would thus be a range, rather than a critical threshold. All curves passing to th left of it would mean winged individuals, all curves passing to the right wou mean wingless ones, but intermediate curves would mean the production true intermediate individuals, which would show greater or less approximatio to the winged state depending on the concentration of inductor at the critic time.

Conversely, mosaic individuals would occur if the critical time of determination ere replaced by a range or period, as in Fig. 46B. Here the critical threshold is e same, but moments of determination are different, and the curve immediately the right of the full "winged" curve would mean the production of individuals hich had Oc, Wm and Sn, but no wings. Slower accumulations of the inductor uld produce individuals having complete Sn, but nothing else.

These two cases are combined into one in Fig. 46N, where there are overlapping imulation ranges, but periods of determination which do not overlap. The econd curve from the left would mean the production of individuals which had lly formed Wm and Sn, but only partial Wg and Oc. The third curve from the ft would mean the production of individuals which had partially formed Wm nd Sn, and no Wg or Oc. The right-hand curve is that for wingless individuals. here would thus be both mosaic and intermediate types. And this is what does tually occur in aphid development. Many other cases are possible, and some re given in the papers of Shull & Stiles.

I shall conclude this section with a few words on the possible biochemical eaning of the condition of competence. Although we have few facts by which guide our thought, we can at any rate envisage certain reasonable possibilities. rising out of Wigglesworth's work on the moulting hormone in bugs (see p. 465), me the suggestion that there may be in the cell as many enzyme reaction chains there are competences; in this case the inductor might be a co-enzyme which ould give one such enzyme reaction chain speed priority over the others. nother suggestion, made to me in conversation with L. Rapkine, is that for ery competence there might exist a specific protein with its own specific recursor, like trypsin and trypsinogen. The inductor would then be a very inute quantity of the protein itself, which would "seed" the system, and bring out the formation of the characteristic protein by auto-catalysis. A third ssibility, in view of the work of Needham, Shen, Needham & Lawrence on osin and adenylpyrophosphate, is that an inductor may be a substrate, and at during the unavoidable reaction which will follow its entry into the com- tent cell, the physical configuration of the enzyme protein may itself be anged. In any case, determined differentiation will be due to the direction of e cell metabolism into a specific path.

2·16. The liquidation of the entelechy

The preceding analysis differs very deeply from Driesch's own interpretation his fundamental experiments[7]. As is well known, he regarded them as affording oof of vitalism, that is to say, of the effective guidance of the embryo's evelopment by a non-material agency. To some it may seem that his views are w of purely historical interest. In my opinion, however, they represent a ndency which has often recurred in the history of scientific thought, a tendency base a pessimism about the power of scientific analysis on a scholastic and dialectical logic. Only those who have well weighed his arguments are fully titled to accept the hopes and promises of true physico-chemical embryology.

It will be remembered that reference was made above to the invariable factor wholeness, *E* (see p. 101), which is involved in the formation of two whole embryos from two blastomeres which, left to themselves, would only have fo one. “It was not without design”, wrote Driesch,[*] “that I chose the lett to represent this. Let that factor in life phenomena which we have shown t a factor of true autonomy be called ‘entelechy’, though without identifying doctrine with what Aristotle meant by the word ἐντελέχεια. There is, how at work a something which bears the end in itself, ὃ ἔχει ἐν ἑαυτῷ τὸ τέ The (Drieschian) entelechy was then defined as intensive manifoldness (*inte* *Mannigfaltigkeit*) as opposed to the extensive or visible manifoldness w becomes evident as development proceeds.[†] All order in morphogenesi exclusively due to the action of entelechy.[‡] The entelechy does not exist in s (space-time), but acts into space,[§] it is therefore not localisable at any poin space-time.[||] Lacking all the characteristics of quantity, and being incapabl measurement, it cannot be a form of energy,[¶] and it cannot therefore affect first law of thermodynamics.[**] Its definition was finally admitted by Dries himself to be “a system of negations”.[††] He could, however, describe its ac as that of suspending possible reactions,[‡‡] one or more of which would other proceed in its absence, and in this sense it could be called an “arranging agent

It seems astonishing to us to-day that Driesch should have had recourse soon in his analysis to a conception so obscure in all its particulars, so ou harmony with the natural products of the scientific method, so certain to c the door to further experimentation, so resembling the Galenic δύναμις “faculty”.[||||] We cannot see to-day why it was necessary to place the intens manifoldness of the egg outside the physical world, in view of the tremend complexity which the colloidal constitution of protoplasm must involve. E at the atomic level there would be ample room for it. It must have b evident enough, even in the last century, before the work of Wo.Köhler[¶¶] and school, that, when a magnet is divided into two, we are not left with one no pole and one south one, but that an immediate “regeneration” of the wh pattern takes place, and that we have two fields of force, similar morphologica to the original one, but smaller. The particles of the magnet are not, as it we “determined” to form part of any given pole, but can form part of eith according to their position in the whole. This analogy only needs tra position to the more complicated realm of biological colloids (see below, p. 12

Again, the *Verschmelzungsversuche*,[***] where two eggs are fused together, one normal embryo results, have their analogy in the long-known phenomen of convergent crystallisation (*Sammelkristallization*). Spatially separated sm crystals commonly unite themselves into large ones of the same structure. S more striking is the behaviour of the ammonium oleate crystals of O. Lehmann and Przibram[5], for these soft forms, if side by side, join up together, passing through all the stages corresponding to *duplicitas* greater or lesser in animals.

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[*] Driesch, H., *Gifford Lectures*, p. 106.

[†] *Ibid.* p. 245.

[‡] *Ibid.* p. 154.

[§] *Ibid.* pp. 254, 298.

[||] *Ibid.* p. 299.

[¶] *Ibid.* p. 256.

[**] *Ibid.* p. 257.

[††] *Ibid.* p. 300.

[‡‡] *Ibid.* p. 261.

[§§] *Ibid.* pp. 292. In this connection it is important that the teleological significance of entelechy was greatly diminished in the following decade by the discovery of self-differentia and the realisation that all embryos pass through a stage, introduced by the action of organisers, where no regulation is possible. The entelechy thus deserts its post when the wor one-third completed. Embryologists were accordingly led to question whether it had ever b there at all.

[||||] See the valuable edition of Galen, *On the Natural Faculties*, by A. J. Brock.

[¶¶] Already in 1877 Pflüger and the botanist Vöchting had seen the significance of the ma analogy for life phenomena.

[***] Accomplished for echinoderm eggs by Driesch[3] and for those of the newt by O. Mangol Seidel. Mosaic eggs, when fused, may give larvae containing two notochords, two nervous syste etc. (v. Ubisch[4] on ascidia; Ranzi[8] on cephalopods).

Driesch's pessimism was often expressed. He did not wish, he said,[*] to discredit a thorough and detailed study of osmosis or colloid chemistry in their relation to morphogenesis. "But these investigations never give us a solution of the problem." Or again:[†] "Atoms and molecules by themselves can only account for form arranged, so to speak, according to spatial geometry, they can never account for form such as the skeleton of the hand or foot." But he touched an important point when, in discussing the limits of entelechial action, he granted[‡] that "it may depend on some very unimportant peculiarity in the consistency of the protoplasm that the isolated blastomere of the ctenophore egg is not able to restore its simple intimate protoplasmic structure into a small new whole". We know now (as the narrative of Huxley & de Beer[2] well shows) that whether the rearrangement of materials needed for regulation around the main polar axis can take place or not is a matter largely dependent on the viscosity of the protoplasm —an eminently physical factor. The distinction between mosaic and regulation eggs, therefore, can partly be defined in terms of internal friction.

The intensive manifoldness above the atomic level in the uncleaved egg generally receives to-day a formulation in terms of the gradient concept, Child's great contribution to biology.[§] The polarities of the egg, brought into being partly by its chance position in the ovary, partly by the point of entry of the spermatozoon, or other similar factors, involve a system of gradients describable in a three-dimensional net of co-ordinates. Quantitative differences in activity along the gradients lead to qualitative differentiation; localisation is due to relative position on a gradient. Even the best cases of harmonious equipotential systems possess limitations unrealised by Driesch; thus the single blastomere isolated from the first four of the sea-urchin regulates because it possesses the full extent of the gradient (cf. pp. 477, 480), for if it is divided transversely, or if the original whole egg is divided transversely (equatorially), no perfect embryo will be formed (Hörstadius[1]; Plough; Schaxel[1]; Conklin[4]). Perfect equipotentiality therefore depends on the possibility of separating a system into parts without segregating regions of different potencies (cf. p. 140).[‖]

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[*] Driesch, H., *Gifford Lectures*, p. 63.

[†] *Ibid.* p. 101.

[‡] *Ibid.* p. 266.

[§] So much has been written on biological gradients that it is hard to give a guide to the literature. The subject will be referred to again below in the discussion of fields (p. 129). The value of Child's views on the "metabolic" nature of the gradients will be found discussed in another place (p. 605).

[‖] It is of some interest that Driesch's argument, though leading to such unmechanistic conclusions, is fallacious precisely because it adopts such mechanistic premises. To these it *adds* entelechy. The fact that a heap of cells (*summenhafte Gesamtheit*) becomes a whole, in spite of any desired removals and injuries, can only be explained by the entelechy hypothesis, says Driesch. But the premise is not correct; the regulating egg is not a heap of cells (*summenhafte Beieinander*), but an organised system. Regulation can only occur if the system is in a position to reconstitute its original pattern after the removal or shuffling of its cells. The unorganised heap of cells is an abstraction which does not enter into the question, and can be banished from discussion. But if it goes, the entelechy goes with it (Bertalanffy, article in *Erkenntnis, loc. cit.* p. 368). In 1927 Driesch[9] is still writing: "Der Organismus kann zwar in jedem Moment seines Daseins seiner materiellen Seite nach als chemisch-aggregative 'Maschine' gekennzeichnet werden, aber seine andere Seite, der Maschinenbenutzer, darf nicht vergessen werden." This is the purest Cartesianism; it was Descartes who introduced the practice of calling organisms machines, and then postulating transcendent mechanics to drive them.

Driesch's proposition* "that a system, in the course of becoming, is una to increase its manifoldness of itself" raises, of course, the whole of the age-l controversy between the preformationists and the epigenesists.† But Woodg points out that an organised entity having components standing in inter organising relations to each other *must* have its degree of multiplicity increa if (1) the number of components is increased, (2) the complexity of the relati in which those components stand to one another is increased, (3) the intrin patterns of the components become different from one another. Thus, granted possibility of spatial repetition of a pattern and the possibility of "differentiat division" and "histological elaboration", there is no need to appeal to agents any kind. If we do not need to invoke the entelechy to account for cell-divisi this alone will suffice in principle, since this process must yield an increase multiplicity in succeeding slices of the "division hierarchy". "Neither t preformationists nor the epigenesists knew anything of spatial repetition pattern or of histological elaboration, since such processes occur only in livi cells, and there was therefore nothing in the experience of the early embryologis to suggest that such things occurred." Here Woodger goes to the bottom of t embryological problem in its biochemical aspect, for our aim must be to discov how it is possible for the formation of duplicate protein (and other) molecul to occur. *The mass-production of replicas is the issue.* Whether it occurs by son form of template method, or by the scission into units of an endless protein chai we have at present no idea.

In his acute analysis of causation, Woodger[2] has another argument with an im portant bearing on the theory of the harmonious equipotential system. If tw entities are manifestly (i.e. apparently) non-different, we cannot infer that the causal predecessors are intrinsically non-different. This is what makes caus analysis so difficult, because it means that there will always be an element of doub in the assumption that the two causal predecessors are intrinsically non-differen That two entities are *not* intrinsically different is *always* an assumption. Thus, i the case of pluripotency, it is argued that, since any given monad in the original eg can become any given part of the resulting embryo, therefore the spatial parts o monads of such eggs are intrinsically non-different. In that case it is impossibl that differences can subsequently arise in development, since the environmen cannot be alone responsible. Hence we are driven to conclude that an entity, th entelechy, which is not a spatial part or monad of the egg, is "at work" producin the differences required for further development. Now it seems clear that suc arguments can never be decisive on account of the principle that intrinsic non difference is always assumed, and consequently it is always open to an unbelieve to say that such experiments do not exclude the possibility of intrinsic difference between the parts concerned *which are not revealed when they are in isolation*. Or, i more biological terminology, the properties of a given monad other than its fate ma well be different according to whether it forms part of a two-cell system, or o one of the two cells in isolation.

The meaning of the phenomena of pluripotency has recently been th subject of an interesting polemic between Mirakel and Wermel. Mirakel purported to bring a proof of the existence of the entelechy logically better than Driesch's, but Wermel had little difficulty in showing that his argument was vitiated by a failure to take account of the dominant position which one part of the egg or egg fragment (the organiser region) has over the remainder. Mirakel considered that better support for the Drieschian hypothesis could be derived from the effects of "*Verlagerungsversuche*", i.e. experiments in which the primary blastomeres of the egg are shuffled at random together and yet give a normal embryo, rather than from the effects of fusion, amputation, or isolation. Some non-spatial agency must be acting upon the shuffled blastomeres in a marshalling manner. But Wermel showed that this conclusion was only true if the material factors which determined the fates of the parts had to act on each one independently and separately; it was not true if one part could determine the fates of the rest. This would then happen no matter what chance arrangement of the blastomeres had taken place. And indeed we know that without the presence of an organiser in the isolated fragment, there can be no "harmonious equipotentiality".[*]

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* Driesch, H., *Gifford Lectures*, p. 319. This is clearly put forward in his *Der Begriff der organische Form*, pp. 42 ff., where we find: "Grundsatz I: Wie im rein Logischen die Folge nicht mannig faltiger an (logischem) Inhalt sein kann als der Grund, so kann sich auch im Laufe des Werder der Grad der Mannigfaltigkeit eines Natursystems nicht 'von selbst' erhöhen. Wer das zulasse würde, würde den Begriff der Kausalität als einer Analogie zur Konsequenz, verletzen." But this unexplained extension of formal logic to natural phenomena justified? If it is wrong, Driesch whole theoretical system is wrong.

† See my *History of Embryology*, ch. 4.

In conclusion, a word may be said of the remarkable animistic and anthropomorphic tendency noticeable in Driesch's concept of an "arranging agent". For centuries science has struggled to rid itself of the remains of popular demonology.[†] When Cambridge got its first Professor of Chemistry[‡] (in 1703), acids were male and alkalies female; minerals grew, like plants, from seeds; slaked lime protested by giving out heat; and solid bodies cleaved to themselves in cohesion because they preferred the touch of the tangible more than the feeble contact of air. The entelechy belongs shamelessly to pre-Boylian biology. The work of the logistic positivists, Carnap, Schlick and Neurath, clearly illustrates this.[§] Their purification of scientific terminology convinced them that the majority of biological concepts, such as cell-division, growth, regeneration, etc., could be reduced to incorporation in the "physical language", i.e. in language based on direct experience. This was not the case, however, with words like "entelechy", which are incapable of formally correct definition, and hence can only occur in meaningless statements.[‖] Schlick's prediction that in the future no more books will be written about philosophy, but that all books will be written in a philosophical manner, implies that the concept of entelechy will be searched for in them in vain.[¶] And the judgment of another philosopher on the entelechy is

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[*] The whole of this argument was foreseen in a somewhat abstract way by Woodger[1]. This is a summary of his reasoning: The possibilities before us in the matter of embryonic development are (1) a Weismannian nuclear preformation; (2) a cytoplasmic preformation; (3) environmental differences during development; (4) appeal to a Drieschian transcendent principle; (5) rejection of the causal postulate. Of these (1) and (3) are excluded as decisively as anything can ever be by experiment. Even Driesch shrinks from (5). No help is forthcoming from (4). Only cytoplasmic preformation remains. "Would it be possible to appeal to just enough cytoplasmic preformation to give us our main axes and the primary germ-layers, and could we thenceforwards appeal on a large scale to relational properties?" (in which organiser phenomena would be included). This is certainly the outcome, in general terms, of modern embryology.

[†] See the interesting article of J. G. Gregory, *The animate and mechanical models of reality.*

[‡] John Francis Vigani, see E. S. Peck.

[§] See Carnap, R., *The Unity of Science*, and the trenchant essays of H. Winterstein (*Kausalität u. Vitalismus vom Standpunkt d. Denkökonomie*) and of M. Hartmann (*Die methodologischen Grundlagen d. Biologie*).

Carnap, R., *Überwindung d. Metaphysik durch logische Analyse d. Sprache.*

[¶] In Driesch's most recent writings[10] (1935, 1937), he assures us that in his vitalism the existence of formative stimuli was never denied, and retires to the impregnable position that life *is* organisation and that the action of organisers in development must itself be organised.

worth quoting. "There is no magic in mind as such", writes Broad, "which
explain teleology. A mind does not account for anything until it has wit en
to have designs and will enough to carry them out. If you want a mind that
construct its own organism, you may as well postulate God at once, for i
cannot perform such a feat, it is hardly likely that what has been hidden from
wise and prudent will be revealed to entelechies."

2·17. Regional differentiation

We must now return to the Induction process and analyse it a little furth
The classical method of testing whether a given material is able to perform
induction is to place it in the blastocoele cavity of a young gastrula (the *Einsteck*
technique of O. Mangold; see Fig. 62). In such circumstances an induction occ
as a reaction between three factors; the graft, the overlying ectoderm of the host, a
the host's organisation centre. During the reaction, the host ectoderm underg
a process of determination to become part of an embryonic axis, which of
includes, besides neural tube, mesodermal organs such as notochord and somit
This determination, and thus the induction as a whole, can only, as we have se
above (p. 112), occur if the ectoderm is in a reactive state; if it is too young duri
the application of the inducing stimulus, no effect will be produced; while if t
ectoderm has already been determined to be something else, such as epiderm
for example, the inducing stimulus cannot alter that determination. In oth
words, the ectoderm must be competent for the primary organiser. In th
classical type of induction experiment, two processes, or rather, two aspects
the same complex process, can be distinguished. Roughly speaking, these tw
processes can be called (1) the determination that an embryonic axis shall
developed, and (2) the decision as to the regional character of that axis.

An induced embryonic axis often has a "regional" character, i.e. it is a he
or a tail or a section of the trunk; some definite slice of the body. Now it is ea
to find out, by vital staining or in other ways, what should be the region
character of the implanted organiser. If we compare this presumptive region
character of the graft with the actual regional character of the induced axis,
find first of all that the induced axis nearly always includes a larger region th
the presumptive fate of the graft would have led us to expect (Spemann[13] for t
newt; Waddington & Schmidt for the chick). Isolated pieces of the organis
region have, in fact, a tendency to regulate themselves to form a whole. We sh
see later (p. 283) some striking examples of this. Secondly, we find that t
determination of the regional character of the induced axis may be due to the gra
i.e. the regional character of the axis may be exactly the same as the presumpti
character of the graft. Thus Spemann[13] showed that organisers with the presumpti
fate "head" can induce heads even in the posterior parts of the host body in t
newt. Waddington[3] described a similar case in the chick, in which, moreover, t
anterior-posterior axes of the host and the induction were opposed. It is therefo
clear that sometimes at least the grafted organiser can determine the region
character of the induced axis as well as its presence. But this does not alwa
occur. If we return to our inspection of different cases of induction, we fin

hirdly, that the regional character of the induced axis may not be the same as he prospective fate of the graft. Thus Spemann[13] showed that "tail" organisers grafted into the head region of the newt gastrula induce not tails, but heads. He avoured the suggestion that this was due to a particular kind of reactivity in the ead ectoderm, but Holtfreter[5] was able to show clearly that no such difference xists between the reactivity of head and tail ectoderm. Holtfreter and Waddington ccepted Spemann's alternative suggestion that in such cases the regional haracter of the induced axis is dictated by the host's organisation centre.

In the classical type of induction we are, then, confronted with two deterinations, that of *presence*, and that of *character*. Needham, Waddington & Needham suggested the term *Evocation* for the first type of determination, and *ndividuation* for the second.* In the ordinary induction, one of these deterinations, evocation, is always performed by the graft; while the other, individuaion, is performed by the graft and the host working together, either in a o-operative or an antagonistic manner. We shall see later on (p. 271) that this istinction has proved of considerable value in discussing the new knowledge of rganiser phenomena. Evocation is the stimulus to neuralisation, mediated by chemical substance or substances, acting upon the competent host ectoderm. Its exact histological effects have yet to be elucidated with absolute clearness. Individuation, on the other hand, is the process which builds the neural tissue so ormed into a nervous system of which the head end is distinguishable from the ail end. It may well be that chemical substances are involved within the individuation process also, but up to the present time it has not been possible to rove their existence. Primarily individuation is thought of as a "field" phenomenon.† It follows that from the implantation of single chemical substances r fractions one would not expect to get well-formed secondary embryos. As will e seen later (p. 277) the implantation of dead tissues usually leads to the formation chaotically arranged organs and tissues, while the implantation of single emical substances or fractions leads only to neural tube formation.

While the exact delimitation of the processes is still not possible, some conderable progress has been made (see p. 278), but the discussion of it must be ostponed until the chemical data have been further described. The question is, hat is the character of a neural axis which has been evoked but not individuated?

While the word "evocation" has had a long but undistinguished literary history, "individuation" iginated in philosophy and passed into biology a long time ago. Commentators on Aristotle, ich as Alexander, Simplicius and Themistius, and later the scholastics, discussed the nature of principle of individuation which differentiates particular objects from others of the same pecies (see Ross' introduction to Aristotle's *Metaphysics*, p. cxv). Hence the word appears in eremy Taylor. Apparently it was S. T. Coleridge, the philosopher-poet, who in his *Theory of ife*, posthumously published in 1848, introduced it into biology. "I define life", he wrote, as the principle of individuation, or the power which unites a given *all* into a *whole* that is resupposed by its parts" and he spoke of the assimilation of the "lower powers" such as cohesion, asticity, magnetism, electricity, and chemistry, into the more highly organised living whole. cannot find that either Herbert Spencer, in his *Principles of Biology*, or Child, in his *Individuality Organisms*, added much to Coleridge. In any case, the word "individuation" is here used a much more restricted and well-defined sense (see Glossary).

Spemann, in his 1921 paper ([10], p. 568), was the first to associate the field concept with the rganisation centre.

There were three possibilities: (*a*) that it would consist of a neural tube acc panied by notochord and somites in their proper relative positions, but that whole structure would be "sausage-like", one end being indistinguishable f the other (see Fig. 47), (*b*) that the axis would be much less definitely arranged, v an abnormal cross-section, or (*c*) that the induced material would consist me of histologically differentiated neural, chordal, and mesodermal cells, with any definite arrangement at all. The second of these possibilities is now kn to be the most nearly true.

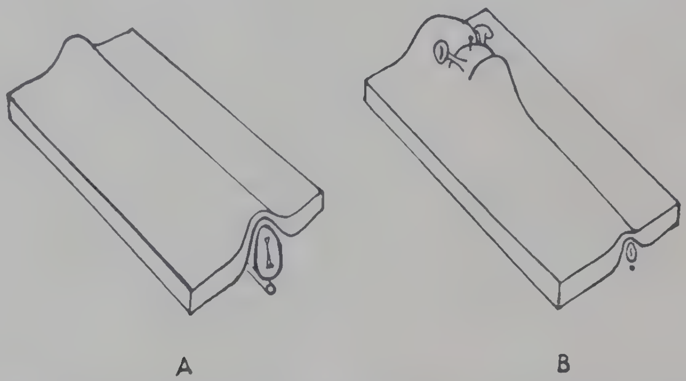

Fig. 47. Diagram illustrating the concepts of Evocation and Individuation. A, neural axis with regional differentiation; B, neural axis with regional differentiation.

The notion of the Individuation Field had previously arisen from the expe ments of Waddington & Schmidt, in which induction of the primitive streak birds by heteroplastic grafts had been studied. In the section on Region Differentiation (2·391), this work will be more fully described; here it is on necessary to outline the concept of the individuation field which was develop in it. It was found that an isolated organiser tends to rearrange its structure as to include all regions of the embryo. Thus, "an organiser whose presumpti fate was to become the mesodermal part of the middle region of an embryo m rearrange itself so as to become the mesodermal part of a whole embryo.... A organiser tends to rearrange the regional structure both of itself and of any tiss lying near it in such a way as to make that tissue part of a complete embry This is then a true unit-building activity of the organiser, which works, not b controlling the process of induction, but by modifying its products." Th activities of the organisation centre could thus be stated as follows. The are, on the one hand, more or less autonomous processes of induction neural plate, etc. (Evocation) by which the organiser can prepare from i surroundings all the types of tissue necessary for the formation of a comple embryo. These processes are not under the direct control of a unit-building forc as can be seen by the occurrence of homoiogenetic induction (see p. 154 But in point of fact the amounts of the various sorts of tissue which are normal formed by these processes just suffice for the production of one embryo. Mea while a process of a different type, which is expressed in the individuation fiel is in control of the regional character of these tissues, and attempts to build the into one complete unit. Waddington & Schmidt described the individuatio field as follows: (1) it is strongest near the embryonic axis and falls off in intensi towards the edge of the area pellucida, (2) it is regionally differentiated alo the antero-posterior axis of the embryo, (3) more anterior parts dominate ov more posterior parts.

The question may be raised whether the action of the individuation field ecessarily depends on the presence in it of its active primary organiser. This s now under study by Abercrombie[3].

2·18. Field phenomena

The conviction that the magnetic field has a part to play in guiding our ideas bout morphological patterns is now of some seniority.* I pointed out earlier hat it fulfills the criterion of divisibility of pattern which was needed for embryoogy (p. 120). Now if an analogy is a true one, the course of scientific thought ill be greatly facilitated; thus Wrinch[1] has referred to the fundamental progress n electrostatics and current electricity due to Clerk-Maxwell's genius in seeing ormal resemblances between the conditions to be satisfied in those subjects and hose satisfied in already solved problems belonging to other subjects. The nasterly apparatus developed by Laplace and Poisson in the treatment of lasticity became available in this way for electrical theory. Can we then obtain nowledge of biological phenomena by means of our knowledge of magnetic henomena? In other words, is there an identity of formal characters in the two ases? Many, especially in recent times, have thought so, notably Weiss[4]; Gurwitsch; and Rudy. Schultz has caustically remarked that fields without entres, without measurable field strengths, and with no obvious lines of force, annot invite comparison with the fields of physics. But this criticism is hardly ustified, for in William Gilbert's time (1600) certain phenomena of magnetic fields

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As a matter of fact it goes back to light-mysticism of Persian and Jewish origin. The Kabala, hich was studied by all seventeenth-century biologists, led in one case to the extremely interesting ork of Marcus Marci, of Kronland, a Czech. His *Idearum operatricium Idea*, published in 1635, as a mixture of purely scientific contributions to optics, and speculative theories about embryoogy. Thus he explained the production of manifold complexity from the seed in generation by an alogy with lenses, which will produce complicated beams from simple light sources. The rmative force radiates from the geometrical centre of the embryonic body, creating complexity it losing nothing of its own power. Monsters originate from accidental doubling of the radiating ntre, or from abnormal reflections or refractions at the periphery (cf. mirror reduplications, cessory organisers, etc.). Marcus Marci, in his forgotten work, thus links together the following ends of thought: (*a*) the old Aristotelian theory of seed and blood, (*b*) the new Cartesian tionalistic mathematical attitude to generation, (*c*) the new experimental approach, in his work optics, (*d*) the cabbalistic mysticism of light as the fountain and origin of all things; finally, his brilliant guess of centres of radiant energy, (*e*) he anticipates the field theory of modern nbryology. Cf. my *History of Embryology*, ch. 2.

The only parallel to this occurs, it seems, in a quarter far removed from Marci at Prag, but qually devoid of influence upon contemporary thought, namely, the *De Motu Animalium* of orelli, the founder of the iatro-mathematical school. Chiarugi gives an account of the chapter n generation (Pt. II, ch. xiv). Its interest is that Borelli compares the semen to a magnet arranging on particles in a field of force. In Harvey, too, a reference to the magnetic field can be found. n the discourse on Conception (1653, p. 539) he says: "The Woman or Female doth seem, after he spermatical contact in Coition, to be affected in the same manner, and to be rendered prolifical, y no sensible corporeal Agent; as the Iron touched by the Loadstone, is presently endowed with he virtue of the Loadstone, and doth draw other iron-bodies unto it."

In the eighteenth century, of course, traces of this are easier to find. Thus from Bourguet, one f the saner ovistic preformationists, may be quoted (1789): "Le mécanisme Organique" which orks in generation "n'est autre chose que la Combinaison du Mouvement d'une infinité de Molécules étheriennes, aëriennes, aqueuses, oléagineuses, salines, terrestres, etc. accomodées à des stèmes particuliers, determinés dès le commencement par la Sagesse suprème, et unis chacun une Activité ou Monade singulière et dominante, à laquelle *celles qui entrent son système sont bordonnées*." (Italics mine.) (*Lettres Philosophiques etc.* Amsterdam, 1729.)

were known although no method was available for measuring field strength. concept of "biological field" does give a powerful aid to the codification o *Gestaltungsgesetze*, the rules of morphogenetic order. As complex compo (in Roux's phrase) they must first be ordered before they can be analysed.*

The field concept, however, has suffered greatly hitherto by an insuffi accuracy of definition, leading in particular to an uncertainty in the attribu of activity to the fields. For some thinkers the fields have been purely descri and symbolic, performing nothing, but permitting us to use a shorthand nota as it were, for morphological form—for others, the fields have been acti arranging entities, performing morphogenetic work. It is of the first importa as Waddington[8] has pointed out, that this confusion should be cleared up.

For Gurwitsch in the main the fields are geometrical symbolism, yet from t to time he speaks of their having an action or an influence.† For Weiss[4] the fi are more active.‡ He draws three main conclusions about them. First, certain amount of material is removed from the domain of a field, the remain of the field manifests in due course the same pattern which it would norm have given in larger size (equipotential sea-urchin eggs, regeneration of amphib limbs). Secondly, if unorganised but organisable material is introduced into field domain, it is incorporated in it (regeneration of limbs, avian developme Thirdly, two or more fields can fuse to form a larger one (fusion experime on invertebrate eggs).§ A similar position is taken by Waddington[26], largely the results of his experiments on induction in the chick embryo. The "Individu tion Field" is a term expressing the tendency of an organiser to rearrange t regional structure both of itself and of any tissue lying near it in such a way to make that tissue part of a complete embryo. In the chick, for example, a pie of neural plate in the process of being induced in ectoderm lying somewh within the host's individuation field may follow one of three paths: (1) it m become fused with the host and the two built up into a composite neural pla (2) it may develop as a separate structure, sharing the regional character of t contiguous parts of the host, (3) its neuralisation may be suppressed, and it m be worked into the host in the form of body ectoderm. The first and second pat are the most usual.

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* It should be noted that there is an intimate relation between the field concept and the theor transformations of d'Arcy Thompson (see p. 531). This would be more generally realised if Thompsonian method had been applied to the change of form during ontogenesis. That partic do tend to orient themselves on progressively distorted co-ordinates is one of the most essen facts expressed by the term "field".

† His definition is: "Als Feld wird hier ein Raumbezirk verstanden, in welchem durch Angabe der Coordinaten jedes beliebigen Punktes auch die Gesamtheit der Einwirkungen auf in betreffenden Punkte befindliches Objekt in eindeutiger Weise festgesetzt wird." *Loc. cit.*, f paper in *Archiv f. Entw.* p. 392. Rudy repeats this.

‡ Cf. his definitions on p. 24 of his *Morphodynamik*.

§ On p. 293 of his *Principles of Development* the criteria are fuller. A field is (1) bound to a mate substratum, (2) an entity, a pattern, not a mosaic, (3) heteroaxial and heteropolar, (4) able include recognisably separate districts, (5) able to maintain its pattern when its mass is reduc (6) able to perpetuate its pattern when completely divided into two or more pieces, (7) able maintain its pattern when its mass is increased, and to fuse with similar pattern entering with new material if the axial orientations are favourable.

But the amount of thought which has so far been devoted to the concepts of eld and gradient is hardly yet sufficient to enable them to bear the load of facts hich is often placed upon them.* The gradient concept is of the two the less undamental, for a system of gradients radiating from a centre would constitute field, and probably no gradient exists only in two spatial dimensions. In this onceptual structure, then, we find that the term "field" is applied to at least wo quite distinct things. In the first place, it is applied to morphological and re-morphological domains of the character just described in Waddington's ords: i.e. wholes actively organising themselves. Here it may not be illegitimate o use such expressions as "the field is a region throughout which some agency s *at work* in a co-ordinated way",† or "the individuation field *sees to it* that"‡ omething or other happens. But, in the second place, there is another kind of egion also to be borne in mind, i.e. regions "possessing a general determination or the production of certain structures and undergoing progressive regional pecification of detail". These, since they only imply *location*, should perhaps be nore properly called "districts".§ We shall return to consider them in more letail later (p. 286).

The situation is, of course, complicated. In respect of any given organ, for nstance, there are to be considered (1) the district from which it normally levelops (the presumptive district), (2) the district from which it will develop if he presumptive district and the surrounding tissue is extirpated, (3) the regions, solated parts of which will develop into the organ if transplanted into its district, 4) the region from which the organ can be formed by a specific or non-specific timulus, etc., etc. We need a new vocabulary for these entities just as much as ew experiments.

Another, possibly fruitful, way of distinguishing between fields and districts ay be derived from a remark of Weiss in his *Morphodynamik*. At a given point a vector in a field, a field force possesses (*a*) a given quality, (*b*) a given direction,) a given intensity. In a field proper, such as the individuation field, we could y that every point will possess a different value for each of these three factors, t in a district only the two latter ones will vary; the first will be the same roughout, e.g. limb or heart. Weiss himself, however, did not distinguish etween fields and districts.

How can a morphogenetic field be defined? Perhaps it is best thought of in rms of instability. Thus, according to Waddington[8], a field is a system of order ch that the position taken up by unstable entities in one portion of the system ears a definite relation to the position taken up by unstable entities in other ortions. The field effect is constituted by their several equilibrium positions. his description is in accordance with the conception of development as a series f successive decisions regarding the fates of parts mediated by a succession of rganisers or evocators, chemical substances acting as morphogenetic hormones.

See the critical review of Huxley[12].

† Huxley & de Beer[2], p. 276.

Huxley & de Beer[2], p. 319.

I reserve the term "area" for the regions of fate-maps. See Glossary.

Instability (high potentiality) is continually giving place to stability (rest potentiality). A similar thought has been expressed by Whyte: at any m the components of the factors mathematically describing the equilibrium re form a continuous spatial function with simple symmetry properties ch teristic of the system.

On the other hand, since we know that the primitive layers of cells of embryos are built are usually indifferent, in the sense that their material m used for the construction of almost any part, stress may be laid upon the known fact that behaviour is a function of position in the whole. Two pr molecules, identical save for their position in the whole, will take part in quite different structures. Thus, according to Floyd: if to every point of a re of space can be assigned a definite value of a variable whose values are descri functions of the organism, so that the properties of the point become a fun of position in the region considered, the region can be called a field. In all t questions the principal conceptual difficulty for the biochemist arises from fact that the cohesive and organisational forces seem to act on what may be ca the supra-chemical level. We can as yet hardly form a picture of the wa which the chemical affinities, the colloidal forces, and the large-scale mecha factors, are integrated in development to produce the morphology of the c pleted animal.

In any case, it is doubtful whether there is anything in the field concept and above that of Function, in the mathematical sense. Thus, in an electrost field one has simply the idea of the totality of values of the (potential) funct associated with a certain set of points. For convenience the equipotential li or curves on which this function is constant are then often drawn in. The along which the function changes with maximum rapidity may also be drawn But the equation contains all this information in itself. Would it not be possi therefore, to arrive at some such equation if we knew the nature and proper of the organiser substances involved, their speeds of diffusion, etc., and also geometrical distribution of competence or reactivity? In the near future we hardly hope for such complete knowledge.

2·19. Conclusion

We have now considered the principal concepts necessary for the understand of morphogenetic stimuli in embryonic development. *Dependent differentia* implies the *determination* of the fate of a part by *induction* from another p *Self-differentiation* is the response to the induction stimulus, which may *primary*, *secondary*, etc. (of first or second grade, etc.) depending on the tim which it acts in development, but the response can only be given by tis *competent* to give it. Normal induction involves two factors, *evocation* *individuation*, the former being the histological change produced by the act of a chemical substance, and the latter the regional organisation which invol in all probability, field forces, though the action of further chemical substan is likely. When self-differentiation has done all it can do, the stage of *functio* *ifferentiation*[*] follows, and the activity of hormonal and neural factors effects a *integration* (Weiss[16]) of the organism.

·2. Mosaic eggs and their biochemistry

As has already been stated, the distinction between mosaic and regulative eggs, hich is not an absolute one, really means a difference in the time at which the etermination of the main features in the individuation field is accomplished. *Iosaic eggs* are those in which this determination has taken place before ertilisation and cleavage[1]; *regulation eggs* are those in which this determination loes not take place until about the time of gastrulation. To the former class elong the eggs of most invertebrate groups, such as worms and molluscs; to the atter belong the eggs of echinoderms and vertebrates. Since it is in regulation ggs that the induction phenomena underlying development can best be studied, nd since our most complete knowledge concerns the eggs of amphibia, the ucceeding sections will be devoted to an account of the development of this class f animals. Problems of wider significance will there be dealt with as they arise, nd only after discussing the chordates shall we return to the invertebrates, specially the echinoderms and insects.

In the meantime something may be said about the mosaic eggs, those which re fully determined already at the time of fertilisation (see the review of Penners[2]). n recent years, two veteran embryologists of the American school have conributed particularly valuable additions to our knowledge in this field. In 1905 onklin[1] described three different kinds of cytoplasm in the egg of an ascidian, *Styela*, a typical mosaic egg; (1) yellow material, which later forms the muscles f the "tadpole", (2) a slate-grey substance, which later forms the endoderm cells, nd (3) the transparent cytoplasm, which later forms the general ectodermal vering of the body. Not until 1931, however, did he publish the results of ntrifuging experiments[3]. Here it was found that strong centrifuging would mpletely dislocate further development. Chaotic differentiation followed; in ig. 48, for example, the notochord and endoderm are on the outside of the ass, with a stratum of neural tissue between them. All imaginable types of islocation were produced; in contrast with the echinoderm egg, which, as is so ell known, can be centrifuged severely without effect on subsequent developent, save an unusual distribution of yolk material among the blastomeres.[†]

The variously coloured regions of the mosaic egg were termed by Conklin[1] ctoplasm, endoplasm, myoplasm and so on, in accordance with their fate, and he cell inclusions (oil-globules, mitochondria, yolk-platelets, etc.) were called 'organ-forming substances". This phrase[‡] in later years fell into much disfavour,

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[*] Little will be said of functional differentiation in this book as we are quite ignorant of its iochemical aspects. A good monograph on functional differentiation is badly needed; meanwhile ference may be made to the short review of Maschkovzev.

Disorganisation of mosaic eggs by centrifuging has been not infrequently reported, e.g. Clement ecently on the mollusc *Physa*.

The phrase should now perhaps be abandoned since we speak of the chemical substances active 1 embryonic induction as "morphogenetic substances", i.e. by stimulus given to competent cells. Veiss[16] suggests "organ-specific areas", but we need the term "area" for fate-maps.

since it was evident from centrifuging experiments (Morgan & Lyon; Mo
& Spooner, etc., see Morgan[4]) that not the heavier or lighter cell inclus
themselves, but the cytoplasm normally containing them, was the impo
factor in the already determined differentiation. Such cytoplasmic region
mosaic eggs correspond to those parts or regions of regulative embryos w
have been determined to form a certain organ or tissue, but have not yet actu
done so. But they must be labile before fertilisation, because in 1938 Da
succeeded in bisecting unfertilised ascidian eggs meridionally. After fertilisat
both halves developed, giving almost normal larvae of reduced size.

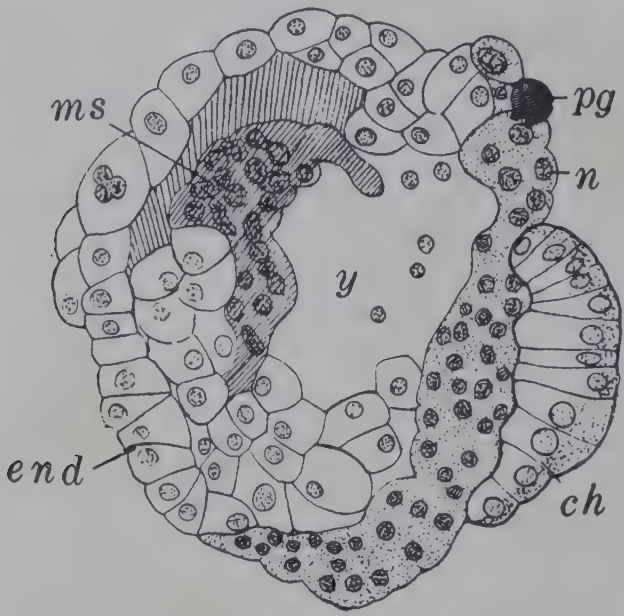

Fig. 48. Eversion of ascidian embryo produced by centrifuging. *ch*, notochord; *n*, neural tissue; *end*, endoderm; *ms*, muscle cells; *pg*, pigmented sensory spot; *y*, yolk.

Another typically mosaic egg is that of the annelid *Chaetopterus*. Though t
first work of E. B. Wilson on this egg[1] was done as early as 1882, he published
1929 some very interesting experiments[3] in which he centrifuged the eggs of t
worm at the beginning of the maturation period. They stratified, broke, and ga
fragments of different kinds, some hyaline, some yolky. On being fertilised
parthenogenetically activated these fragments showed a close approach to norm
development, including even the characteristic rather complicated spiral cleava
and gave normal trochophores. Yet after fertilisation, this egg, in the norm
course of events, would show typical mosaic development. This was a cle
demonstration that the primary determination process occurs much earlier
mosaic than in regulative eggs. It should therefore be theoretically possible
find something analogous to the primary organiser in mosaic eggs, acting at t
time of the departure of the polar bodies, but this will be an extremely diffic
problem, as the removal of parts from a whole without general injury is mu
harder in the case of a single cell than in the case of a gastrula. This problem h
analogies with that raised by the work of Hämmerling (see p. 417).

It was natural that embryologists should ask themselves the question wheth
anything further could be found out about the different cytoplasms of mos

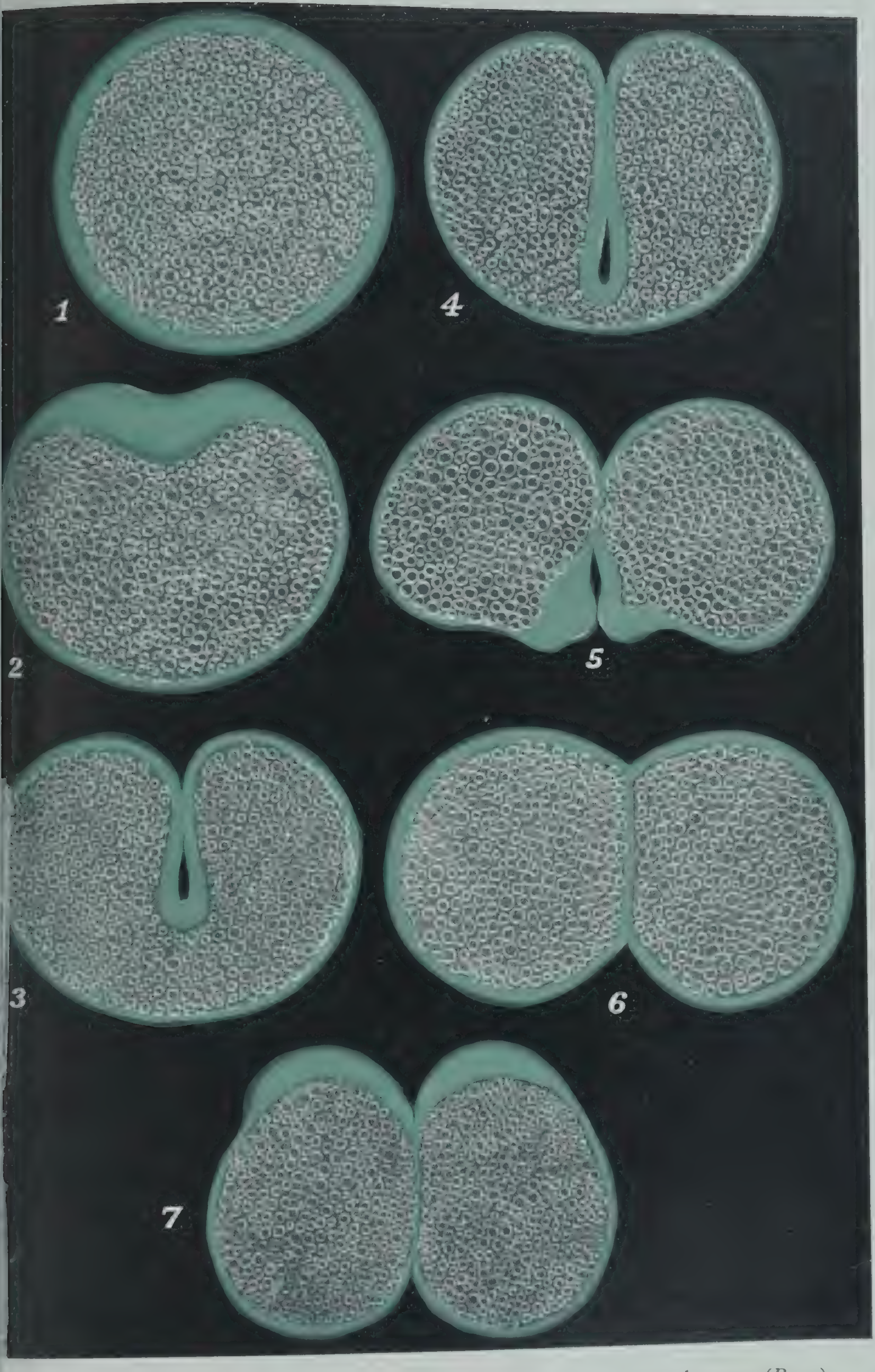

Fig. 49. Differences between cytoplasmic cortex and interior in a ctenophore egg (*Beroe*) seen under dark-ground illumination. For later cleavage stages see frontispiece.

eggs. This has led to a considerable mass of work which is rather difficult to appraise biochemically. Apart from certain specific histochemical work, to be referred to later, the line of least resistance has been to study the appearances which the eggs present after staining by vital dyes. There has been a tendency to interpret the effects as due to differences of *p*H inside the cells, but as reliable *p*H ndicators rarely penetrate the cell-membrane, and as the dyes that do penetrate t are not usually reliable *p*H indicators, statements in the literature have to be accepted *cum grano salis*. On the other hand, this literature cannot be entirely neglected, for if a vital stain shows a quite different colour in different regions of a mosaic egg, or in different blastomeres, for example, we can at least say that *some* differences must exist between the cytoplasms of these regions. It must be admitted, however, that we cannot even say that these differences are chemical, for the birefringent and non-birefringent portions of a mammalian muscle fibre react differently to vital dyes, although the evidence goes to show that they differ not chemically but simply in the different arrangement of the myosin fibrils. Moreover, nobody knows the chemical significance of different colours taken up by vital dyes in cells. On the other hand, if it can be shown that polar or asymmetric differences in vital staining appear only in mosaic eggs and not n regulative eggs, this supports the view that determination means a localisation* or *Sonderung* of specific cell elements into different protoplasmic regions. That this is the case is no doubt the most important result of the work here to be described.

It was opened by Spek's examination of the mosaic egg of the ctenophore, *Beroe ovata*, under a microscope with dark-ground illumination. In these conditions, the cortex of the one- and two-cell stages showed a brilliant emerald-green colour (see Fig. 49), and when followed through later development, this green aterial went almost entirely into the crown of micromeres, which later form the ctoderm (by epiboly). With vital dyes, the difference between the thick cortex nd the cytoplasm within it was well marked; thus with neutral red the cortex as yellow and the interior pink, while with nile blue the cortex was pink and he interior blue.

In later work, Spek[3] gave particular study to the eggs of the polychaete worm, *ereis*. That of the species *dumerilii* possesses a natural indicator, lemon-yellow n the alkaline side, violet on the acid; this showed a bipolar *p*H difference soon fter the extrusion of the polar bodies, the endoderm-forming vegetal pole being cid and the ectoderm-forming animal pole being alkaline. This agreed with the nts taken up by neutral red (yellow at animal pole, red at vegetal pole), and to ome extent validates conclusions drawn from neutral red on eggs of other species. The *p*H "gradient" became intensified during cleavage. The study of the pecies *limbata*, the eggs of which do not have the natural indicator, confirmed he previous conclusions (Spek[5]), and a similar *p*H localisation or polarity as found in *Chaetopterus*. On the other hand, when echinoderm eggs were nvestigated by similar technique, no separation of plasms was to be found (Spek[6]).

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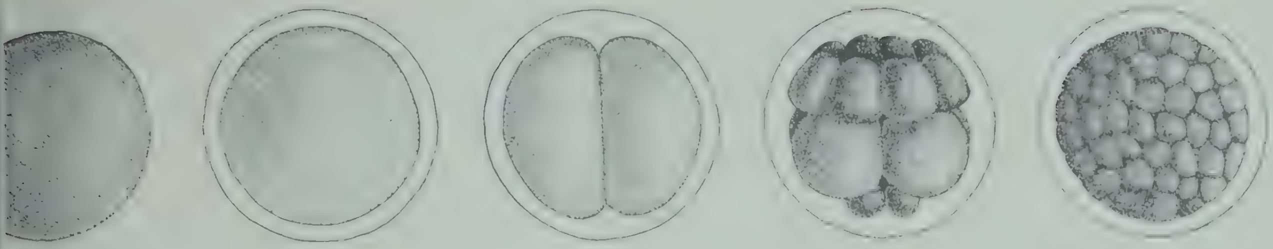
* I should like to use the word "segregation" here, but to do so would be to invite confusion ith Lillie's theory of differentiation, criticised on p. 113.

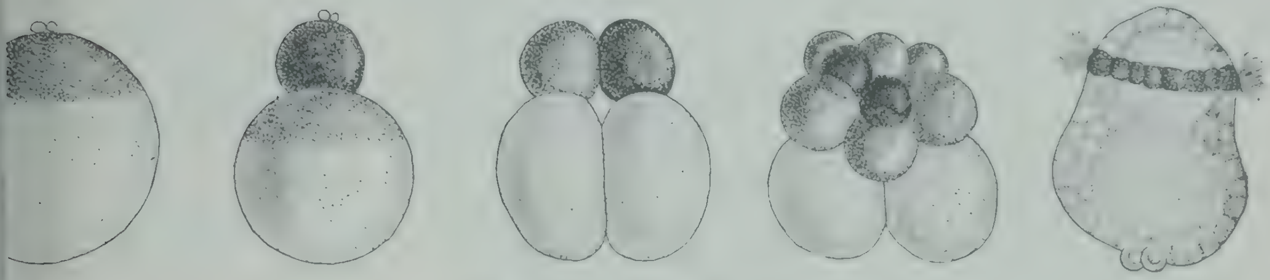
A slight difference in vital staining between cortex and cytoplasm wa that could be seen until nearly the time of gastrulation. Thus, at the 64 stage, the vegetal pole cells no longer colorise with cresyl violet or nile sulphate, while the animal pole cells still do so. Under abnormal conditions, h ever, such as the use of hypertonic salt solutions, a polar localisation was observa all the acid material (as judged by methyl red) going to the animal pole. significance of this is not yet clear, but the general distinction between mosaic and regulation eggs was confirmed in a striking way not by experiment but direct observation. In Spek's most recent work[8] by vital staining on the am blastula of the sponge *Sycandra*, however, there is a discrepancy, for inte localisation was shown, although it has been found that some sponge embryos quite regulative (Teissier[2] on *Sertularia*).

Spek's line of attack was extended by Ries & Gersch to the egg of a moll (*Aplysia limacina*), typically mosaic. In this form, polarity arises at the time the formation of the polar bodies. Soon a marked stratification has taken pla at the animal pole there is a cytoplasmic region with mitochondria, then a b of fatty yolk-platelets, then a ring of granules and finally the mass of large yo globules. If the egg is placed in reduced methylene blue, the animal pole regi turns blue first, but later on all the dye accumulates in the vegetal region, a in the ring of granules. Correspondingly, janus green is reduced to the r derivative throughout the vegetal part of the egg, but not at the animal po The first cleavage cuts across the zone of granules, but the micromere contai mostly animal pole material and the macromere mostly vegetal pole materi The position at the two- and six-cell stages is shown in Fig. 50 (opp. p. 146); ea blastomere gives a different reaction to the dyes used (vital blue and toluidine blu Again, Fig. 51 (opp. p. 146) shows how macromeres reduce janus green while micr meres do not; the latter also oxidise reduced methylene blue much more th the former. Trypan blue is taken up only by the micromeres and their descenda tissues. From the data obtained, an approximate value of pH 7·8 was given f the animal pole cytoplasm and about 6·0 for the vegetal pole material, but, althou a pH difference may be admitted to exist, the values must be regarded as doubtf Subsequent work by Gersch & Ries and Cohen & Berrill extended the study the localisation processes to ascidians such as *Ascidiella*, *Phallusia* and *Ciona*, a to worms such as *Pomatoceros* and *Herpobdella*, with results substantially t same as those already described. Echinoderms, such as *Arbacia* or *Sphaerechin* however, gave no sign of localisation of plasms with any of the dyes or reage employed. The contrast is well seen in the illustration, in which the oxidation reduced methylene blue is compared between the various species (Fig. 52); all cases, except the echinoderm, there is to be found a marked localisation of t property to one or two blastomeres.

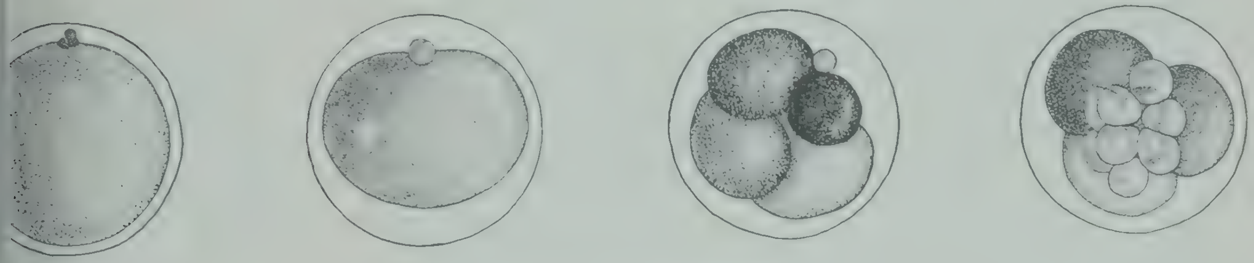
To what extent are the substances present in the separated plasms identi with those which may be stratified in definite order by centrifuge experiment Raven[6], and later Ries[3], showed that they are the same by centrifuging mosaic eg from ovarian stages before localisation and determination had taken place. Vi dyes then gave the same colours to the various stratified layers as had previous



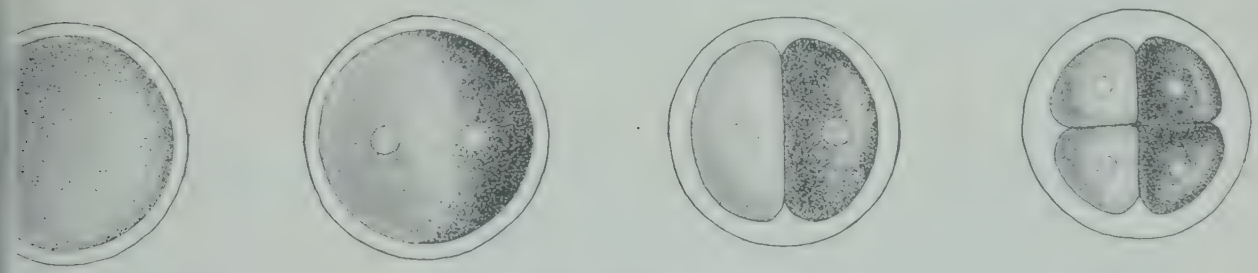
Arbacia (sea-urchin)



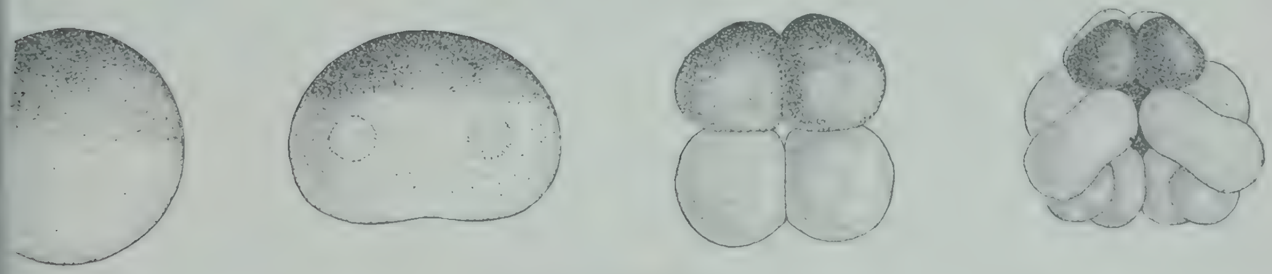
Aplysia (opisthobranch mollusc)



Herpobdella (annelid worm)



Pomatoceros (annelid worm)



Ciona (ascidian)

Fig. 52. Methylene blue oxidation during the cleavage stages in a number of species; all the mosaic types show localisation, the regulative echinoderm does not.

been observed with the separated plasms. The heavy centrifugal material te to be "alkaline", the rest "acid". Raven found little tendency for reorganisa of the egg to take place in accordance with its proper axes after centrifuga (*Chaetopterus*, *Nereis*, etc.), but it sometimes occurred (*Aplysia*).

The subject was given a more chemical twist by Ries[1,4], who investigated distribution of ascorbic acid (vitamin C), fixed –SH groups,[*] benzidine per dase,[†] indophenol oxidase (by indophenol blue formation),[‡] besides the oxida of reduced methylene blue and reduction of janus green.[§]

Except those of *Aplysia*, invertebrate eggs seem to contain little or no asco acid. But in *Aplysia* a marked localisation of this substance was found (cf. Fig. The micromeres get most of it from the outset, save that a small crescentic pa occurs in the macromeres. This they lose altogether, however, to certain descend cells by the 20-cell stage.

The fixed –SH groups were found to increase in quantity after fertilisa in the echinoderm egg (cf. p. 421) but no localisation was to be seen; in ascid eggs, on the other hand, an unequal distribution was found.

The peroxidase reaction, too, is very localised in *Aplysia*, being just as confi to the macromeres as ascorbic acid is to the micromeres. This association w the vegetal yolk material persists into the larval stages. In another mosaic e that of the ascidian *Ciona*, the localisation is exceedingly sharp (see Fig. 54); i confined to the vegetal pole blastomeres, and by the time of gastrulation associated particularly with the muscle-forming mesoderm cells. This is interesti since we can thus identify the "peroxidase"-containing protoplasm with t yellow "myoplasm" of Conklin, and the long-known "organ-forming substances thus receive for the first time, however weakly, a biochemical connotation. In la stages, as would be expected, the tail muscles show a strong reaction, but not oth parts of the tadpole. Moreover, Konopacki[5] has shown that in the eggs of *Clav lina*, glycogen is also confined to the yellow "myoplasm" and its derivatives.

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[*] Ries was under the impression that he was viewing the localisation of glutathione, but as nitro-prusside test was made after trichloracetic acid fixation, it is likely that the tripeptide lost by diffusion and that it was the fixed –SH groups which gave his reaction.

[†] True (thermolabile) peroxidase occurs only in milk. The oxidation of benzidine to a b derivative by hydrogen peroxide in the presence of a catalyst probably means, therefore, presence of haematin compounds (thermostable) in minute quantities. They may of course none the less important for that.

[‡] The formation of indophenol blue (see pp. 566, 569) from α-naphthol and *p*-phenylene-diam in the presence of a catalyst (indophenol oxidase) is the classical test for the oxygen-activat "Atmungsferment" of Warburg. From the work of Keilin & Hartree, we now know that reaction proceeds somewhat as follows:

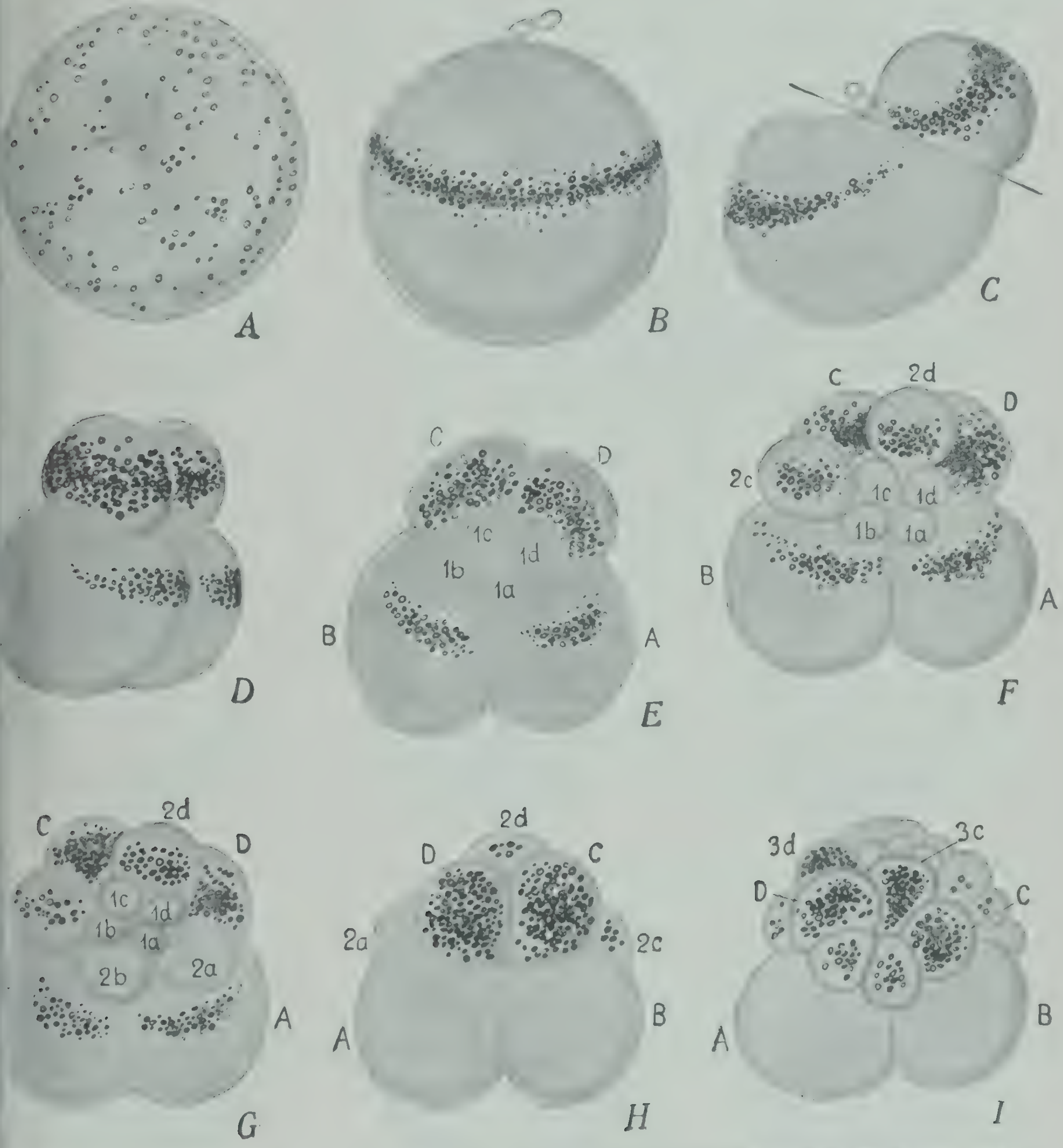
$$\alpha\text{-naphthol} + p\text{-phenylene-diamine} + 4\ \text{cytochrome-C} \xrightarrow{\text{spontaneously}} \text{indophenol blue} + 4\ \text{reduced cytochrome-C}$$

$$4\ \text{reduced cytochrome-C} + \text{O}_2 \xrightarrow{\text{“Atmungsferment” (cytochrome oxidase)}} 4\ \text{cytochrome-C} + \text{H}$$

Absence of colour-formation, therefore, may mean either that the indophenol oxidase is lacki or that cytochrome-C is lacking, or both.

[§] This may well be regarded by biochemists as a "job lot" of processes, but what methods available depends on technical factors and not upon what we would like to know.

The indophenol blue reaction followed, in *Aplysia*, the distribution of the scorbic acid rather than that of the peroxidase reaction. It was confined to the icromeres except for a special crescentic area on the macromeres. The echinoderm egg again stood in sharp contrast (*Paracentrotus*), for while the reaction


A
B
C
C
D
D
C
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1c
1d
1b
1a
B
A
E
C
2d
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2c
1c
1d
1b
1a
B
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F
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1c
1d
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2b
2a
A
G
2d
D
C
2a
2c
A
B
H
3d
3c
D
C
A
B
I

Fig. 53. Localisation of ascorbic acid during the cleavage stages in *Aplysia*. (The numbers indicate the cell-lineage.)

ok place especially strongly, every part of the egg or morula reacted to exactly e same extent. In *Ciona* the reaction was again confined to the yellow "myolasm" and followed this through development. With the oxidation of reduced ethylene blue, three reactions were thus found to be specially associated with e yellow plasm.

Now just as Conklin[3] had shown that development of the ascidian egg is i
fered with by centrifuging, so Ries[3] showed that this holds true of *Aplysia*
and the same is reported of *Nereis* eggs by E. N. Harvey[6].

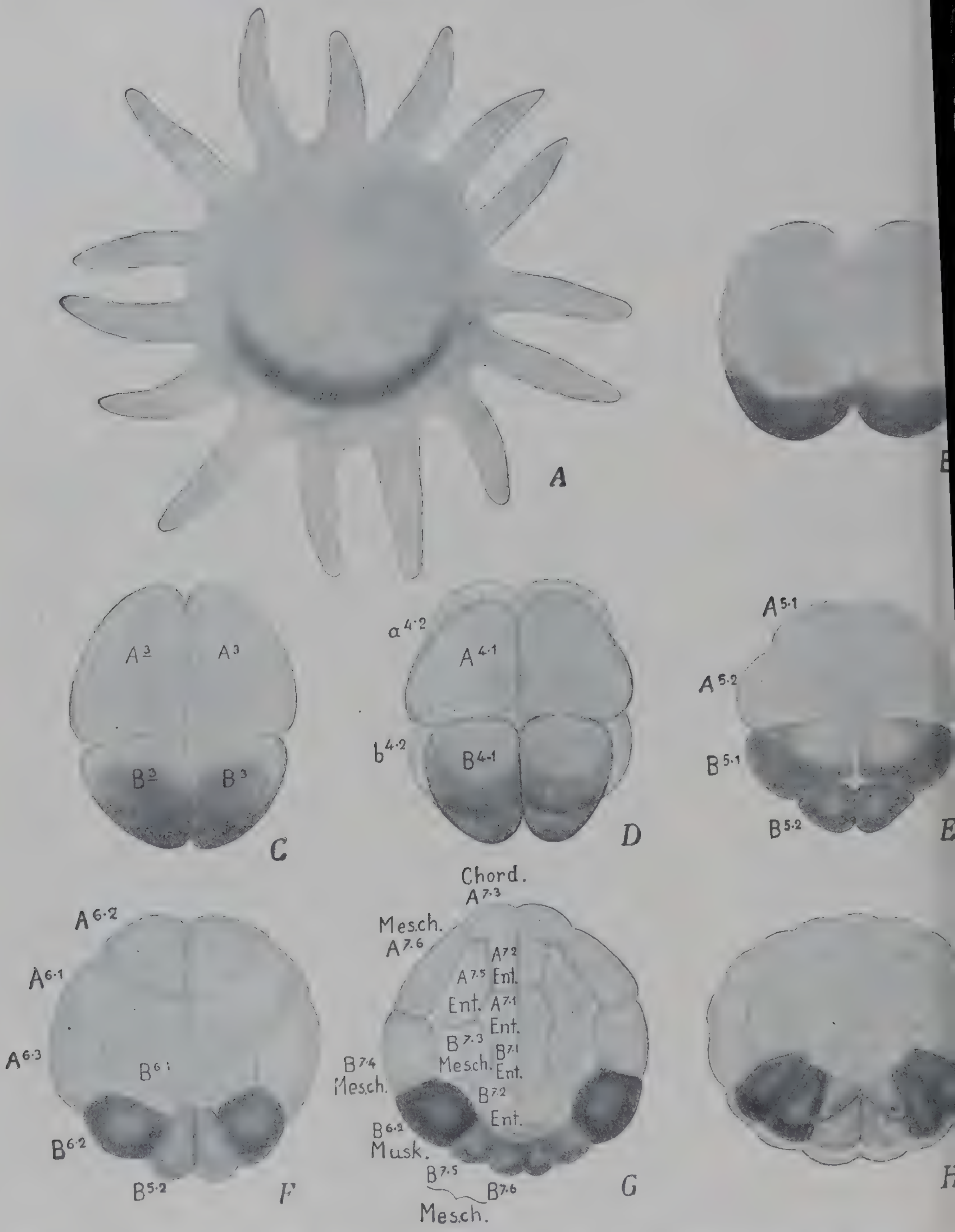
A
C
A3
A3
B3
B3
a4·2
A4·1
b4·2
B4·1
D
A5·1
A5·2
B5·1
B5·2
E
A6·2
A6·1
A6·3
B6·1
B6·2
B5·2
F
Chord.
A7·3
Mesch.
A7·6
A7·2
A7·5 Ent.
Ent. A7·1
Ent.
B7·3
Mesch.
B7·1
Ent.
B7·4
Mesch.
B7·2
Ent.
B6·2
Musk.
B7·5
B7·6
Mesch.
G
H

Fig. 54. Localisation of the peroxidase reaction during the development of *Ciona*. *Ch*
Notochordal material; *Mesch*, mesodermal material; *Musk*, muscle material; *Ent*, endo
mal material. Numbers indicate cell-lineage.

The eggs of nematode worms (studied mainly on various species of *Asca*
present a very special case. Of the first two blastomeres, one, the prosoma

see below), can produce only ectodermal derivatives; the other, only endo-mesolermal (stomodaeum and mesoderm) derivatives. But within these two domains ar-reaching regulation is possible, including single embryos produced by fusion f eggs, and repair of deficiencies. Dependent differentiation and self-differeniation are therefore, as Schleip says (*Determ.* p. 291), combined in nematode evelopment in a peculiar and characteristic way. Correspondingly development emains relatively unaffected even by the severest centrifuging (Beams & King[1]). hen Wottge (in whose paper many references to the previous literature will be ound) examined *Ascaris* eggs from the point of view of plasmatic localisations, ttle of this kind could be seen, e.g. glycogen or lipins in histochemical tests on erial sections. The egg, as is well known, is almost completely impermeable once he membranes have established themselves after fertilisation; there is on the utside a protein membrane, then a chitin one and then a membrane which seems be largely composed of cholesterol.* Vital staining therefore gives no help. *scaris* eggs are also very specialised in that they have relatively enormous acuoles in the cytoplasm, which tend to accumulate at the egg's periphery before he first cleavage. The only visible localisation is a transient accumulation of ranules at the vegetal pole before the first cleavage.

Among the mosaic aspects of the *Ascaris* egg, however, is the fact that at the rst cleavage it divides into a prosomatic blastomere and a progonadal blastomere; ne latter later gives rise to the germ-cells as well as to some somatic material. n the somatic cells deriving from both blastomeres there is a diminution of hromatin at successive divisions from the second to the fifth, but not in those ells from which the germ-cells will arise. This is explained as being due to ne local elaboration at the animal pole of a specific chemical substance, a diminisher", from a precursor localised there; this diminisher, though diffusible, differentially segregated during cleavage so that the nuclei of some cells have undergo diminution, but those of others do not. By centrifuging strongly, leavage, but not nuclear division, is suppressed; the diminisher has therefore ee access to all parts of the egg, and the distinction between prosomatic and ogonadal cells is abolished, for all the nuclei cast off chromatin into the toplasm (King & Beams; cf. the experiments of Moore[7] on the "cleavagebstance" in the echinoderm egg, p. 364).

The general upshot of all the work of Spek, Ries, and their collaborators has en to suggest that in many eggs showing mosaic development† a profound emical heterogeneity of the parts of the egg sets in very early, parallel with the rly determination. In regulative development, on the other hand, a certain mogeneity‡ is preserved until the time of gastrulation. Histochemical and perimental methods confirm each other. The former alone will not carry us uch further, but with the micro-chemical technique which should before long available, it ought to be possible to establish by unimpeachable chemical ethods the differences between the determined areas. It should also be possible do this for areas in regulative eggs between the moment of determination and e onset of histological differentiation.

This may explain the unique resistance to high pressure shown by nematode eggs, 800 atmoheres being needed to stop development (Pease & Marsland).

Not necessarily in all, for little or no localisation was to be found in the eggs of the polychaete rms *Aricia* and *Chaetopterus* with the methods so far used (Ries[1]).

This is, of course, not to be understood as denying the fundamentally heterogeneous colloidal aracter of all egg-protoplasm.

As a footnote to the above, it may be mentioned that according to Gersch
dye trypan blue will distinguish between mosaic and regulation eggs, for
the former (e.g. *Aplysia* and *Pomatoceros*) pursue their development unaff
by it, the latter (*Psammechinus* and *Chrysaora*) are completely ruined, giving
for instance, to chaotic proliferations of ectoderm.

Among the groups of animals which have been omitted from the foreg
discussion, the cephalopods are perhaps the most remarkable. Alone an
invertebrates, their eggs possess so much yolk that cleavage is discoidal, as
in the fishes. In such eggs the fact that the main mass of nutrient yolk diffe
staining reactions or in *p*H from the blastoderm may not mean that a determina
localisation has taken place. That there is such a difference was shown by Sp
who found the yolk (with good indicators) to be strongly acid, and the blastod
alkaline (5·0 against 7·8), with corresponding staining differences. But whe
the cephalopod egg is absolutely mosaic or not is doubtful, as comparati
little work has been devoted to it. The most likely supposition is that it devel
mosaically after the first cleavages (after Naef-stage V), i.e. it is much m
mosaic than the echinoderm egg but slightly less so than those of annelids
ascidians. This may indeed be true of all molluscan eggs, if we may judge fr
the discussions of Ranzi[8], who found cases of *duplicitas cruciata* in cephalop
and yet was able to show by explantation experiments that after a relatively ea
stage full self-differentiation would proceed (Ranzi[9]). Earlier reports in
literature (cited in these just-mentioned papers) of conjoined twin larvae fr
annelid and molluscan eggs had always been difficult to reconcile with a str
mosaic view, and had been explained away as the result of the fusion of two eg
originally separate. But when Penners[1] in 1922 described the production of tw
embryos from the oligochaete *Tubifex rivulorum* after equal cleavage of the eg
cell (instead of the usual unequal cleavage), and when some years later Titleba
found that *Chaetopterus* twins could be produced fairly regularly by compressi
the uncleaved egg, doubt was no longer possible. Later a thorough investigati
by Tyler[1] showed that all kinds of agencies (such as compression, abnormally hi
or low temperatures, centrifuging, and anaerobiosis) will make mosaic eggs g
rise to twins. All that these agencies have in common is that they alter the positi
of the first cleavage spindle. Instead of its ends lying in regions qualitativ
different they come to lie in regions qualitatively alike, and hence when the e
divides, two cells are formed each of which has sufficient of each of the vario
plasms required for complete differentiation. At this stage, therefore, there m
still be much plasticity.

The subject has been placed in a clearer light by the admirable experiments
Novikov on the eggs of the annelid worm *Sabellaria*. In exo-gastrulati
experiments all parts show perfect self-differentiation (in sharp contrast w
what happens in amphibia, see p. 159), and the self-differentiation of parts
unaffected by the transplantation of polar lobes and blastomeres in all possi
combinations, even though the contact between the cells may be shown to be cl
enough to permit of the diffusion of dyes such as nile blue (Novikov[1]). But if
treatment with KCl, cleavage is temporarily inhibited so that both the two fi

lastomeres receive polar lobe material, then twin embryos, perfect in every articular, result with great regularity (Novikov[2]). It may thus be concluded that e polar lobe does contain an organiser or inductor but that it cannot pass across ll boundaries and can only be transmitted at mitosis. Explanation of this fact ould be had if some inductor substances are active in protein combination reasonable possibility; cf. the discussion on p. 205). Its significance is that it rovides a bridge between the diffusible substances of the type of the amphibian rimary evocator and the non-diffusible nuclear inductors described in Section ·67.

Further work will make more precise the exact degree in which each inverte-rate and prochordate egg approaches true mosaic development. In the mean-me there is no contradiction between Spek's bipolar *p*H localisation in the phalopod egg and the morphological results, since, as we shall see later, a ipolar *p*H localisation occurs also in the eggs of teleostean fishes (Spek[4]), here the development is eminently regulative. Localisation, in a word, only has relevance to determination when the cleavage is total.

Before leaving the cephalopods, the possibility of inductive processes in later evelopment ought to be mentioned (cf. p. 325, where it is seen that these occur the later development of the highly mosaic ascidian egg). The cephalopod eye-p, so astonishingly similar in organisation to that of vertebrates, develops, as is ell known, from an ectodermal invagination, not from the nervous system. anzi[4] says that the development of the optic ganglion is a dependent differentiation quiring at first a stimulus from the eye-cup, though later able to proceed naided. Thus the situation in cephalopods would be exactly the reverse of that vertebrates.* The cephalopod eye is particularly sensitive to toxic substances, nd cyclopia readily occurs (Ranzi[3]).

3. Amphibian development and the fundamental principles of morphogenesis

2·31. Description of gastrulation

n order to assist the reader in the discussion which follows, we must first cribe very shortly the well-known, but in many ways still rather obscure, ocess of Gastrulation. Fig. 55 gives a series of longitudinal vertical sections the successive stages of the process.

After fertilisation, the amphibian egg divides itself into a large number of cells the cleavage, or segmentation, period. At the vegetal pole, where the mass of k has accumulated, the cell-divisions, impeded by this material, occur more wly, so that at the time when the solid ball (the morula) becomes a hollow one blastula, with a blastocoele cavity), the vegetal pole cells are very much bigger an those at the animal pole. There is also a difference of pigmentation. The imal-pole cells are always darker than the vegetal ones, so much so in ne cases, such as the common frog (*Rana temporaria*), that the vegetal cells a pure creamy white and the animal ones a dark coppery brown, almost black. the newt, *Triton alpestris*, the difference is rather between two browns, and re are some amphibia, such as the newt, *T. cristatus*, whose eggs have no

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f. the curious effects described by Dragomirov[6], where the amphibian head ectoderm seems nduce a retina in rotated eye-cups.

pigment at all, so that there is little difference between animal and vegetal cells. This has been taken advantage of for chemical colour experiments, a be seen later (p. 203).

The essential happening in gastrulation is that all the marginal zone bet the two kinds of cells, and all the vegetal pole cells, become invaginated enclosed within a ball of ectoderm. The process is seen beginning in Fig

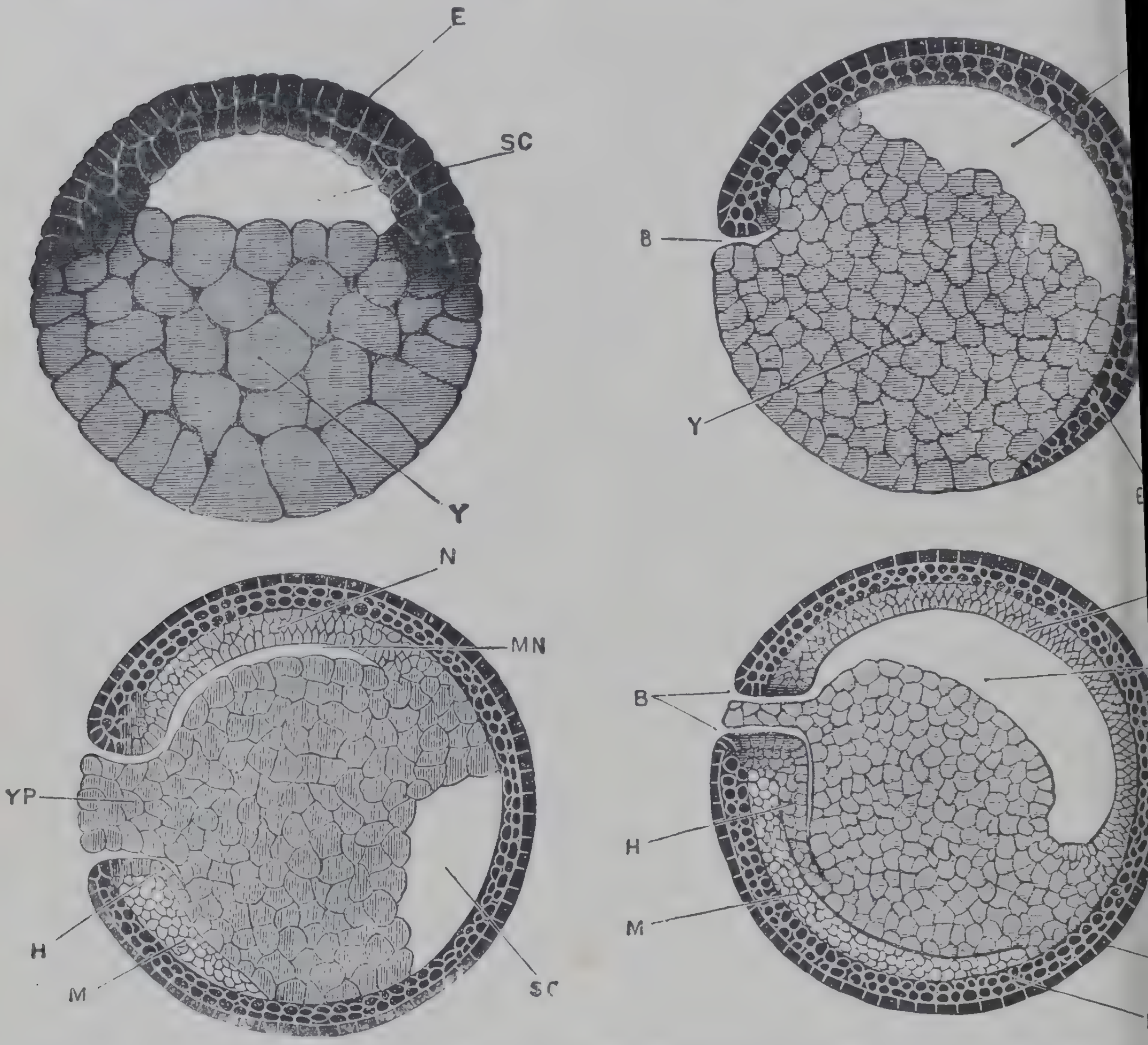

Fig. 55. Longtitudinal vertical sections of the amphibian embryo during gastrulation (f Milnes-Marshall). *B*, blastopore; *E*, roof of the blastocoele; *EE*, *EN*, ectoderm; *H*, ven lip of blastopore; *M*, mesoderm; *MN*, archenteron; *N*, roof of the archenteron; *SC*, bla coele cavity; *Y*, yolk-endoderm; *YP*, yolk-plug.

Animal pole cells creep around the vegetal pole yolk-cells on all sides, but w this difference, that whereas at the anterior part of the egg (*EE* and *EN* in Fig. the cells remain continuous with the yolk-cells at the surface; at the poste part of the egg (*B* in Fig. 55) a groove appears, the dorsal lip of the blastopo Through this discontinuity the great invagination begins, cell material passin at the blastopore to form the roof of the primitive gut or archenteron (*N* Fig. 55). As this happens, the old blastocoele cavity is pushed farther and fart forwards and downwards until it eventually disappears, as can be seen in Fig. At the same time the blastopore progressively closes, passing through a success of shapes, quarter moon or sickle-shaped, half moon, and finally full moon.

ıaterial visible *in* the full moon from outside is the remaining yolk, the yolk-plug *P* in Fig. 55). Eventually the full moon narrows to a hardly visible slit. The ree germ-layers of classical embryology are now formed; the ectoderm, which closes the entire embryo; the mesoderm, which forms the archenteron roof d which has been invaginating also from the ventral lip of the blastopore (see g. 55); and the endoderm, the mass of white yolk-cells. All the layers are ntinuous, and by these formative movements the principal organ systems of e future embryo have reached their definitive positions.*

As the blastopore finally closes, the neural folds make their appearance on the ırsal surface of the embryo, defining the edges of the neural plate, which is aped like a shield, broader at the anterior end than at the posterior.† It is to noted that the material which passed in first through the dorsal lip of the astopore will be incorporated into the future head (actually forming part of the ıarynx), that which passed in next will form part of the trunk, and the last to ter will form part of the tail. The neural folds now move together towards the id-line, forming a narrow groove, the neural groove. When the sides of this se, the neural tube, the primary axis of the future vertebrate animal, is formed. the same time, the archenteron roof lying below divides into five longitudinal ips lying side by side. The centre one of these becomes the notochord, and nce the foundation of the eventual axial skeleton, while the strips to the ımediate left and right of it cut themselves up into a linear series of blocks, the imitive muscle masses, or somites. Finally, the two outer strips spread out on ch side of the body forming the side-plate mesoderm, from which will arise in ıe course the coelom.‡

2·32. The mapping of the presumptive regions

A knowledge of the exact presumptive regions of the embryo before the complishment of the formative movements of gastrulation was a necessary logical eliminary to the study of the results of interference with them. A scheme of normal fates of the parts (a "fate-map") is required if we are to recognise an ıormal fate when we see it. The earlier workers, such as His and Roux[3], who ›reciated the importance of the problem, could only attack it by making small ons in the embryo, but since healing is very active in young amphibian tissue, y made no progress. It was not until the appearance of the two great papers Vogt[2], who applied vital stains to the surface of the embryo, that the problem s thoroughly and definitively solved.§

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he foundations of our knowledge of cleavage and germ-layer formation we owe to the work . E. v. Baer[1, 2] (1828–1835), whose instructive and witty autobiography should not be neglected anyone interested in these subjects.

ıs the result of cinematographic work, Daniel[1] gives a kind of eyewitness description of this ›cess. With proper lighting the archenteron roof can be seen moving forward like a curtain. anges then occur in the presumptive neural area in the following order: (1) the cell-layer thins dially, (2) two streaks of pigment accumulate, (3) at the inside edges of these the neural folds se, extending backward as well as forward. One may be normally several hours behind the ıer.

The yolk-endoderm has also a part to play at this time. On its upper surface, which forms floor of the archenteron, a trough appears which later closes, not unlike the neural folds, into ther tube, the future intestinal tract.

t is interesting that the use of coloured marks in studying space-time changes in living organisms es back to the eighteenth century when Stephen Hales examined the growth of leaves with rks of red lead and John Hunter used madder for following the deposition of bone.

Minute pieces of agar were soaked in the dye solution (nile blue or neutr dil. 1 : 1000) and held against the gastrula for a short time. After the d been absorbed by the cells it will be retained by them for a long time w spreading, so that they remain recognisable and can be followed in movements. The result of marking the outside surface of the early gastr seen in Fig. 56 A and B. In the first of these a row of differently coloured was placed dorsally to the dorsal lip of the blastopore, running in a series the animal pole. As gastrulation proceeded, the series passed downward in at the blastopore, eventually coming to lie in the mesodermal notochord in the archenteron roof, where it could sometimes be seen in transparency thr the outer ectodermal cells. The second experiment showed how marks p all around the blastopore, but at some distance away from it, moved towar

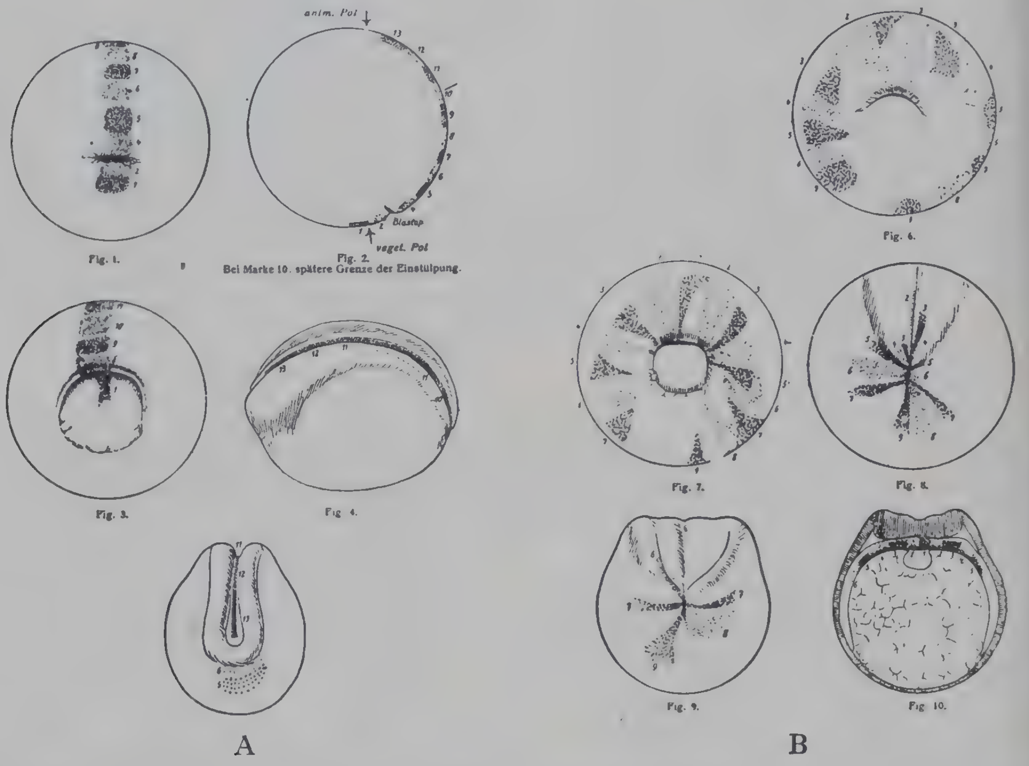

Fig. 56. Movements of stained areas during amphibian gastrulation, showing the cell-strea

and were invaginated in a way reminiscent of coloured liquids being washed d a drain. It is to be noted that the marks reach the dorsal lip of the blastopore f since it is there that the invagination is most rapid and earliest. Later on, howe when the yolk-plug has altogether disappeared, and a slit-shaped blastopore a remains, the marks are seen vanishing into its sides, and even also at its ve lip. By the time the neural folds have formed, hardly any colour is left on outside of the gastrula, but as the cross-section shows, it is readily to be fo again in archenteron roof and side-plate mesoderm.

The resulting map, which, with minor variations, holds good for all amphibia, is seen in Fig. 57. The upper map shows the gastrula seen from left side; the lower one shows it seen from the vegetal pole. Taking the for first, it will be observed that rather more than half the total spherical su consists of material that will not invaginate, of which presumptive epide (lying over the old blastocoele cavity) makes up about one-third, and presump neural plate about two-thirds. The endoderm yolk-cells will, of course, also not invaginate, except in the sense that they will be drawn into the interior of the gastrula when the yolk-plug vanishes as the blastopore finally closes. All the rest of the material will invaginate through the blastopore, the beginning of which is seen at *J*, a point near the old ventral pole but a little farther away from the lowermost point of the egg at its present stage owing to changes in its centre of gravity. At the dorsal side lies the rather large area of future notochord, beneath it and on each side of it the area of the future somites, still farther downwards and ventralwards the future side-plate mesoderm, and filling up the remainder of the space, the future tail mesoderm. The lower map helps in the visualisation, which can, of course, be best attained with the aid of a model.

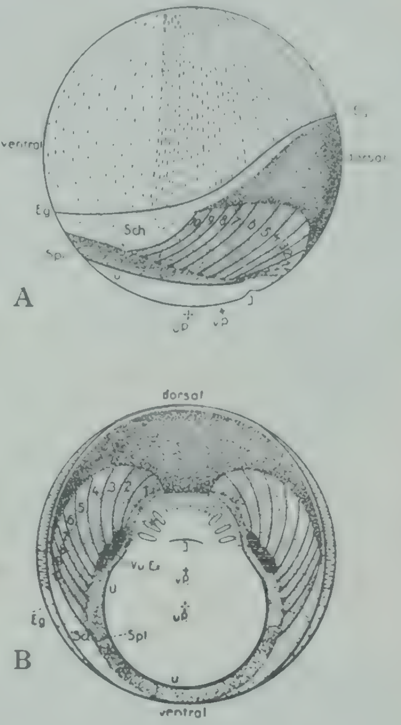

Fig. 57. Vogt's fate-map of the amphibian gastrula. *Eg*, limit of invagination. *Sch*, tail mesoderm. *Spl*, side-plate mesoderm. *u*, endoderm. *Vu.Ex.*, fore-limb. *J*, site of blastopore. *vP*, vegetal pole. *uP*, lowermost point at this stage. A, from the side; B, from below.

It is to be emphasised that we have here a map of the future, a "fate-map", as it were, and that there is nothing to distinguish these various regions, microscopically or to the naked eye, in the early gastrula itself. Whether the regions may be distinguished by any chemical differences is almost entirely a matter for the future, but there are indications that the presumptive mesoderm (the organisation centre) differs from the rest (see p. 203). It is unlikely that any chemical differences will be found among the other, undetermined, reas.

Since the time of completion of this mapping process for the amphibian embryo, it has been confirmed n many forms (e.g. Schechtman[1] in America) and extended to many other ertebrates. From the results general theoretical considerations have been drawn, s by Dalcq, in his monograph; Pasteels, etc. Important though these are for hylogenetic and other problems, we cannot discuss them here, and will refer only o the several maps in the section pertaining to other chordates (Section 2·41).

2·325. The nature of the formative movements

Besides the movement of invagination which has just been described, other movements, equally important, take place during early amphibian development.*

That there must be some inner compulsion to invagination on the part of dorsal lip tissue (*Einrollungstendenz*) is shown by the fact that if a piece of it s transplanted to another place, it rolls in on its own account (Spemann &

One must remember that movements of migration continue to play an important part till late in evelopment, e.g. the wanderings of neural crest mesectoderm (Harrison[18]) and the migration of lacode cells from the acoustico-lateralis rudiment which gives rise to the sense-organs of the teral line in amphibia (Stone[2]); to say nothing of the development of the peripheral nervous stem.

H. Mangold). Moreover, its direction is fixed, and even when the transpl invaginating cells are oriented in the contrary direction to the host embr forces its way in, as Spemann[8] and F. E. Lehmann[3] showed. Even the amou invagination is determined in these isolated pieces, for the blastopore lip young gastrula will force its way in much farther, according to Spemann, an old one. This kind of determination has been called "dynamic determinati as opposed to induction.

The mechanism of this invagination tendency is still extremely obscure. I examines a cross-section of the blastopore lip at its earliest stage, such as in Fig. 58, one sees that the cells at the base of the furrow have a distin bottle-shaped form of their own. These were first described by Ruffini in 1 who attributed to them secretory functions. Several authors (e.g. Goodale; V Wintrebert[3,4]; Daniel & Yarwood) have figured these piriform cells and discu the possibility that they act in a contractile manner. The claim has been mad Wintrebert that by choosing a temperature sufficiently low to inhibit this c traction without inhibiting cell-division (+3° C.), the appearance of the blastop could be held up until the egg was returned to normal temperature. However this may be, it would be of much interest if contractility could be shown to play a part in gastrulation, in view of the results on insect embryos (discussed in Section 2·81) and the chemical evidence (p. 191) for amphibia. Ruffini also showed that the endodermal cells again become bottle-shaped or piriform when the intestinal tract forms as a furrow on the floor of the archenteron.

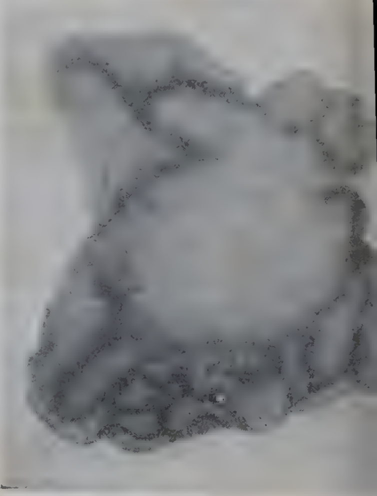

Fig. 59. Expansion of ectod in two ventral halves of trulae grafted together.

The material which invaginates must come from somewhere. It is provided by the vast expansion of the dorsal and animal pole ectoderm, sometimes called divergence or epiboly. A well-known experiment of Spemann's[17] strikingly illustrates this. When two ventral halves of an early gastrula are grafted together, there does not result a smooth-surfaced ectodermal ball containing yolk-endoderm, but a most irregular forr tion, full of lobes and folds on its spreading puckered surface, for the ectode of both pieces is expanding. The "organism" progresses no further, of cou owing to the absence of the organiser centre. Such a formation is seen Fig. 59. Dalcq[6], who has given much attention to the nature of these moveme attributes very plausibly the formation of the amnion in sauropsida and even proliferation of the trophoblast in mammalian embryos to the same ectoder tendency to expansion.

What is really difficult, however, to obtain from the morphologists is a sa factory statement regarding the mechanism of these formative movements. T Huxley & de Beer[2]* speak merely of "streaming movements and displaceme of the various regions of the embryo" without specifying the process invol

* *Elements*, p. 25.

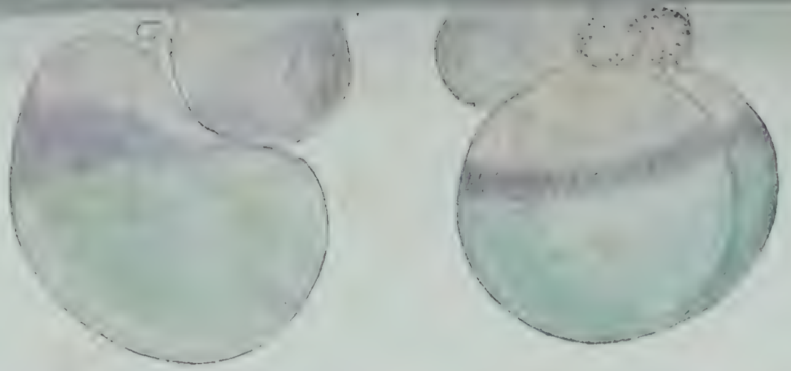

Fig. 50. Staining differences between regions of the embryo of *Aplysia* at the 2 and 6 cell stages.

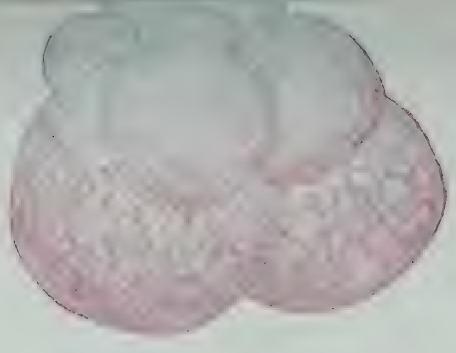

Fig. 51. Staining differences between regions of the embryo of *Aplysia* at later cleavage stages (more intense reduction power of the macromeres).

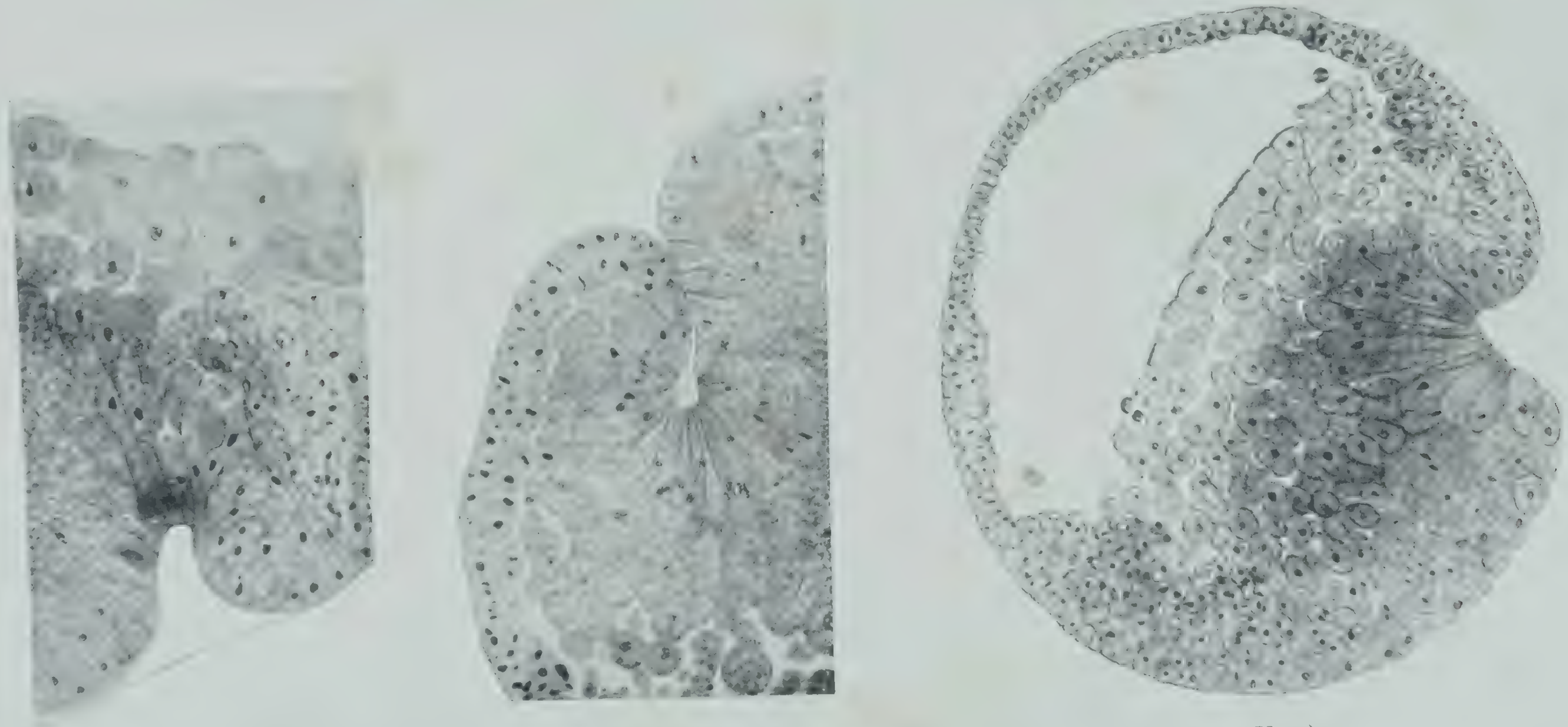

Fig. 58. Sections through the dorsal lip of the blastopore showing the piriform cells (from Vogt).

and Pasteels[9], who has worked a great deal on the movements in many different vertebrates, speaks of them just as "déplacements cellulaires" or "migrations". Vogt himself said little on the nature of the process, but believed that cell-division played a certain part ([2], p. 434). Evidently either cell-divisions, perhaps oriented in a specific direction, or changes of shape of the ectoderm cells must be responsible. These processes need not be mutually exclusive.

On the subject of cell-divisions, as estimated by counts of mitoses, the evidence is rather conflicting, and it is difficult to discuss it without reference to embryos other than those of amphibia (see below, p. 329). We must first admit that changes of shape alone can do a great deal. The most cursory dissections of amphibian embryos suffice to show that the roof of the blastula is much thicker than the ectoderm of the later gastrula, whether presumptive neural plate or that lying over the blastocoele cavity. The latter is often extremely thin, more so in the newt than in the frog. A four times diminution in thickness, which is not out of court, would allow for a very substantial expansion of area. It has, however, been maintained by Wintrebert[5] that there does exist in the marginal zone a centre of active cellular proliferation (in *Discoglossus pinctus*), but his evidence, as may be seen from the reply of Pasteels[7], was unsound,* and no mitotic counts were presented. Pasteels denies that mitoses play any part. On the other hand, mitotic counts have recently been made on the gastrula of the toad, *Bufo cognatus*, by Bragg, with the following interesting results.

		Mitotic index (mitoses/100 cells)
Blastula:	Roof of blastocoele	64·8
	Vegetal pole yolk-cells	44·5
Early Gastrula (crescentic blastopore stage):		
	Dorsal lip of blastopore	58·4
	Cap at animal pole	81·1
	Other ectoderm cells	56·0
	Yolk-endoderm cells	12·7
Late Gastrula:	Ectoderm cells	8·8
	Blastopore lips	8·1
	Mesoderm cells	5·9
	Yolk-endoderm	2·6

his table illustrates the higher mitotic rate of animal pole cells than vegetal pole ells before gastrulation, and the great decline in all areas at the end of gastrulaon. Particularly interesting was the small area of frequent mitoses central to ne expanding ectoderm early in gastrulation. The size of this area, says Bragg, s remarkably small; the ratio of number of cells in it to total cells in the embryo s 1 : 284.

Laborious though work on mitotic counts must be, it would be very desirable o have confirmation of these effects on other amphibia, though in all probability lattening and thinning of the blastula roof plays the greatest part.

The origin of this dynamic determination presents a problem of absorbing nterest. In his discussion of it, Spemann[17] points out† that, although it obviously ccurs much earlier than the material determination mediated by organisers, it

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Wintrebert also spoke of a "mitogenetic" centre, but we may leave this altogether out of ccount (see p. 422).

Embryonic Development, p. 107.

is capable of wide regulatory changes. Ruud & Spemann, for example, shov that gastrulae from which the entire ventral half has been removed will gastrul normally and produce neurulae normally shaped but reduced in size. This mea of course, that material which would never normally invaginate will have to do More recently, Schechtman[9] has found that the areas of presumptive mesode at the sides of the dorsal lip are necessary for its invagination. If they removed or replaced by presumptive ectoderm, the dorsal lip moves outwards form one of the "horns" so often described by the earlier workers, instead invaginating.

Besides the movements already discussed, i.e. invagination of mesoderm ai expansion of ectoderm, there are two others of much importance, longitudin extension (*Streckung*) and dorsal convergence. Longitudinal extension manifes itself in the formation of the tail bud, and occurs in isolation, as Bijtel h shown, when presumptive tail material is explanted. Dorsal convergence appea principally in the approach of the neural folds to each other, and their closur but it is also seen in the concentration of invaginated mesoderm in greate thickness in the region of notochord and somites, and at a still deeper level in th closure of the endodermal furrow at the base of the archenteron cavity to forr the future intestinal tract.

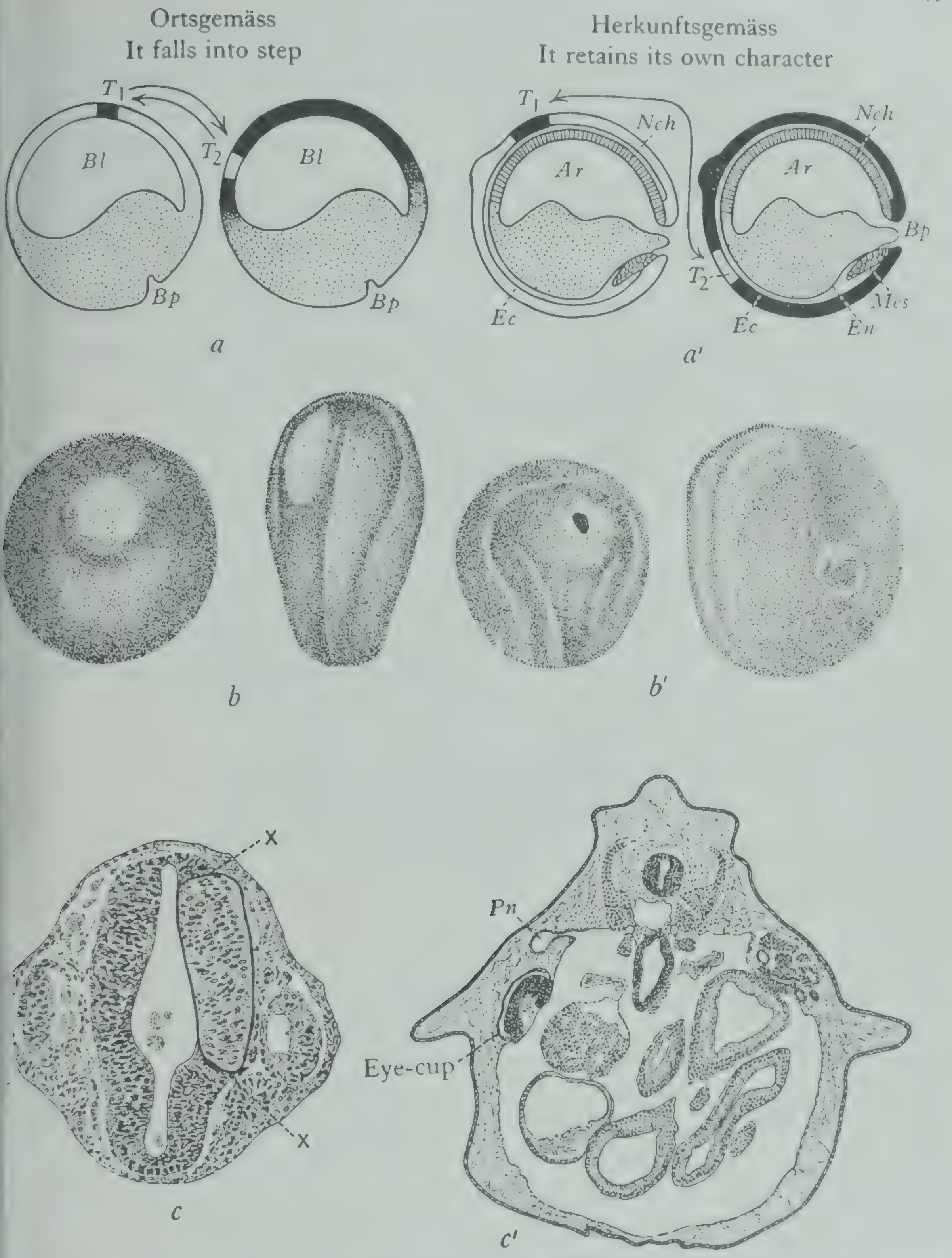
A fuller description of all these movements will be found in the monograp of Hatt. Bragg's measurements of mitotic rate suggest that in these also cell divisions play a part, though it may not be a preponderant one.

Finally, Schechtman[3] and Daniel & Schechtman have described a movemen of yolk-endoderm cells from the ventral pole upwards in the blastula stage o certain urodeles. This "unipolar ingression" seems to contribute to the convexit of the blastocoele floor, but its significance is otherwise unknown. Were these cell to maintain their connection with the cortex of the egg, they would assume th bottle-shape referred to above, and if the piriform processes contracted, gastrulation might ensue.

2·33. The appearance of the vertebrate axis

2·331. Properties of the primary organiser

As has already been mentioned (p. 101) exchange transplantations (*Austauschversuche*) performed on the amphibian embryo by Spemann and his schoo (Spemann[8,9,10]; O. Mangold[1,2,4]) showed that before gastrulation has begur almost all parts of the embryo are interchangeable with other parts. They usec the heteroplastic method, i.e. transplantation between the unpigmented embryo of *Triton cristatus* and the pigmented ones of *T. taeniatus*, taking as their mode the classical work of Harrison[1] on the origin of the lateral line organs in 189 and 1903, about which Spemann[17] was later to write that it taught him "mor than almost any other investigation, not only for technique, but also for th methodically advancing analysis". Fig. 60 shows, first, an exchange betweer presumptive epidermis and presumptive neural plate. Material which woul have formed skin formed brain, and *vice versa*.


Ortsgemäss
It falls into step
Herkunftsgemäss
It retains its own character
T_1
T_2
Bl
Bl
Bp
Bp
a
T_1
Nch
Ar
Ec
Nch
Ar
Bp
T_2
Mes
Ec
En
a′
b
b′
X
X
c
Pn
Eye-cup
c′

ig. 60. Exchange transplantations demonstrating the determination process occurring during gastrulation.

a, *b*, *c*, exchange of presumptive brain with presumptive epidermis in the early gastrula.
a′, *b′*, *c′*, exchange of presumptive eye-cup with presumptive body-wall in the late gastrula.

T_1, T_2, grafted pieces.
Bl, blastocoele cavity.
Bp, blastopore.
Ar, archenteron.
Nch, notochord.
Ec, ectoderm.
En, endoderm.
Mes, mesoderm.
X, *X*, limits of brain tissue formed by graft.
Pn, pronephros.

A difference of germ-layers made no difference to the interchangeab
Presumptive epidermis, transplanted by O. Mangold[1] into the dorsal lip
(presumptive mesoderm), were duly invaginated and formed mesodermal so
and notochord. The converse experiment was carried out recently by Lopasc
who transferred presumptive mesoderm from the blastopore lip to presum
neural plate in an older embryo. Though in most cases, owing to its inhe
stretching tendency, excrescences were formed, there were some quite un
biguous ones in which it became incorporated into the host's neural t
Presumptive mesoderm is thus nearly as competent to receive the neuralisa
stimulus from axial mesoderm as is presumptive epidermis or presump
neural tube. These two types of experiment were later more or less combine
one by Töndury[1] (see below, p. 170).* Evidently the distinction between
germ-layers is a convenient morphological one with little or no physiolog
meaning.†

After the completion of gastrulation, on the other hand, the behaviour
transplanted pieces is no longer neighbourwise (*Ortsgemäss*) but selfw
(*Herkunftsgemäss*). Fig. 60 shows an experiment of Spemann's in which p
sumptive eye region was transplanted to the presumptive body-wall. H
it did not follow its new environment, but on the contrary produced a well-form
eye-cup with retina and pigmented epithelium. The fact that this new formati
was, as it were, looking inwards towards the viscera, may be noted in passing
an instance of that lack of teleological character which is characteristic of organi
phenomena.

On the other hand, it was found that when the presumptive mesoderm o
young gastrula (dorsal lip of the blastopore) is transplanted to presumpti
epidermis or elsewhere, it always sinks beneath the surface, either by tr
invagination or by being covered with ectoderm. "There it does not join in t
development of its surroundings, but sticks to its own direction of developme
Indeed it does more than this. It induces or even organises its surroundin
and builds from its own substance and from that of its surroundings a sm
secondary embryo, which can reach a high degree of perfection." This was t
fundamental experiment of Spemann & H. Mangold. It is shown in Fig.
In this particular case the induced embryo possessed somites, pronephr
intestinal tract, and ear-vesicles. Spemann[8] had already been led to suspect t
existence of some special properties in the dorsal lip of the blastopore, for in
rotations of the entire animal pole roof of the gastrula through 90 or 18
followed by healing in the new position, it had been found that the neural a
always developed from the unrotated dorsal blastopore lip, irrespective of t
orientation of the gastrula roof. Or if a young gastrula were cut sagitta
(longitudinally), and the two right or the two left halves allowed to heal togeth

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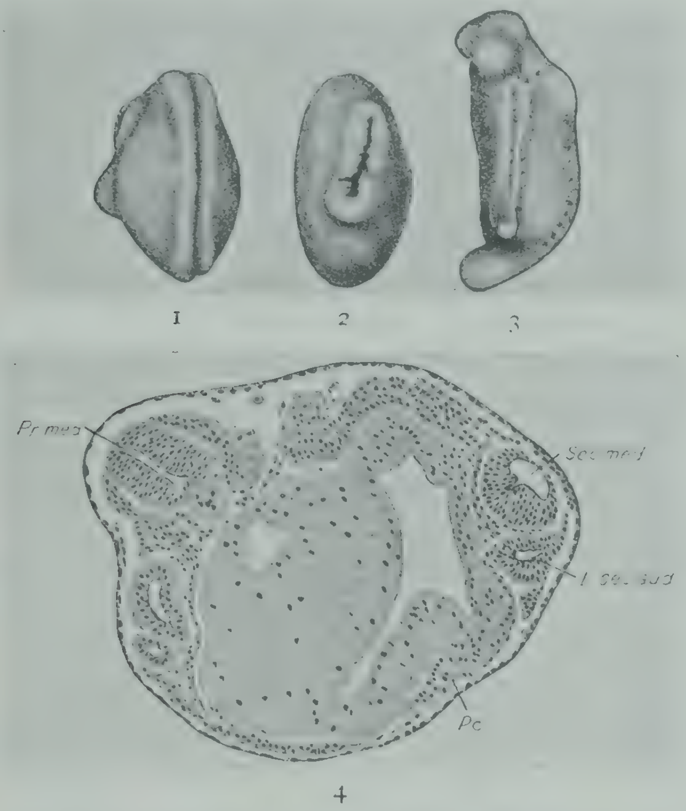
* And indications that chick germ-layers are also interchangeable are not wanting (Rudnic
Rawles; Waddington & Taylor). There is an admirable review on the whole subject by Opp
heimer[9].

† In this connection it is interesting that, according to Brien[1], the neural tube regul
arises during asexual development in ascidians, not from ectoderm but from endoderm
mesoderm.

ouble embryos had been formed, each of the neural axes of the twin proceeding rom one of the half-lips.

"So ergab sich", wrote Spemann & H. Mangold, "der Begriff des Organisa- ionszentrums als eines Keimbereiches, welcher den übrigen Teilen in der)etermination vorangeeilt ist und nun selbst determinierende Wirkungen estimmten Betrages in bestimmten Richtungen ausgehen lässt. Mit der Analyse ieses Organisationszentrums soll durch die hier darzustellenden Experimente in erster Anfang gemacht werden."



61. The fundamental experiment of Spemann & H. Mangold; induction of a secondary embryo by transplantation of the organiser. 1. The host embryo from above. 2. The host embryo from the side, showing the induced neural folds. 3. The same at a later stage. 4. Transverse section showing the two neural tubes. *Pr.med.*, host neural tube; *Sec.med.*, induced neural tube; *L.sec.aud.*, induced ear-vesicle; *Pc*, pericardium.

It is interesting that as far back as 1907 Warren H. Lewis[3] had transplanted the rsal lip of the blastopore, but only to much older larvae, where it developed in cordance with its presumptive fate into notochord and somites, together with me neural tissue, but without affecting in any way the host tissues, which by at time had become too old to retain any competence for neural inductions.

In the ten years that followed the discovery of Spemann & H. Mangold a nsiderable number of investigators carried out that large body of work which s made the development of the amphibian embryo more clearly understandable an that of any other organism, although, as we shall see, the sea-urchin egg tes a good second place. Many researches were devoted to the elucidation the organisation centre as the leader in regulatory processes. Thus Wessel ick together two dorsal halves of young gastrulae, so that the invaginating mesoderms collided, and not being able to do anything else, diverged one to side, the other to the other. In this way a cross-shaped twin was produ and the experimental explanation, in terms of twin organisers, of one of most puzzling of the classical teratological phenomena, *duplicitas cruciata*, attained. Spemann & Wessel-Bautzmann investigated the effects of g excesses or deficits of the material awaiting determination by the organiser. was done by cutting young gastrulae transversely but not equally and then stic together the two large pieces and the two small ones. If the operation is m very early in gastrulation perfect regulation results; if later on, all kinds anomalies are produced. Great deficits in the total amount of presump ectoderm were produced by Spemann[1] and Schmidt[1] in their constriction exp ments already referred to (p. 103); nevertheless, that part of the embryo wh contained the organiser regulated perfectly, so that some material which would normally have become ectoderm must have done so. Conversely, Waddingto showed that considerable regulation will take place when great excesses of p sumptive ectoderm are added to a young gastrula.

Perhaps the most thorough of these regulations was that performed by embryos operated on by S. C. Wang who divided one gastrula sagittally (lon tudinally), another frontally (transversely), and stuck the two different pie together. Since the fused embryo possessed one dorsal half and one left or rig half, it had one and a half organisation centres. Nevertheless, in favoura cases these fused and normal neurulae resulted.

Equally important was the exploration of the gastrula to determine the ex limits of the region possessing inductive capacity and its movements (*Abgre zung*). Spemann & H. Mangold had come near to suggesting that the inducti effect was something to do with the invaginating mesoderm, and soon aft their work the *Einsteckung* method of O. Mangold was introduced.* Using t method Geinitz quickly obtained good neural inductions by implantations dorsal blastopore lip, and Marx[1] followed by showing that the effect could be h equally well with pure archenteron roof. About the same time it was confirm by F. E. Lehmann[1] that removal of pieces from the invaginating dorsal blastopo lip led to very serious deficiencies in the embryo's nervous system. The mo thorough examination of the extent of the inducing area was made by Bautzman who cut the presumptive mesoderm of the dorsal lip into small pieces with ingenious "micro-fork" and implanted them, thus surveying the boundaries the region. His results are shown in Fig. 63, from which it was concluded th most of the presumptive mesoderm possesses inductive power before invaginati (most of the dorsal posterior quadrant of the early gastrula). As the early gastru was used, in which but little invagination can have taken place, it is certain th the inductive power must appear to some extent in the region to be invaginat before invagination has actually begun.

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* As we have already seen, this consists in pushing whatever is to be tested into the blastoco cavity of a young gastrula through a small slit made in its roof (the presumptive epidermi See Fig. 62. When the cavity disappears, the piece comes to lie between the yolk-endoderm a the ectoderm. Thus it has every opportunity to manifest any inductive power which it may posse This method has been of inestimable help as a routine test (see below, pp. 172, 176).

The mesoderm of the notochord and somites had been shown to possess nductive power.* Bautzmann[3] next showed that this was concentrated in the entral notochordal region rather than in the somites or side-plate mesoderm. his line of thought led to a particularly interesting result when Waddington[12] ound that the just-formed side-plate mesoderm in the middle yolk-plug stage an induce the formation of a secondary neural plate when implanted into the lastocoele cavity of a young gastrula. It therefore possesses inductive power. onversely, the dorsal lip of a young gastrula, substituted for the side-plate esoderm just mentioned, induces a neural plate on the flank of the embryo. herefore the flank ectoderm is competent. Yet in normal development no ccessory neural plates appear on the sides of the embryo. This remarkable aradox may perhaps be explained by assuming a quantitative diminution in

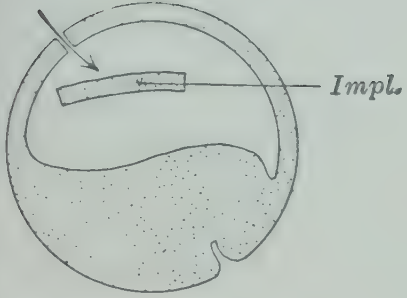

Fig. 62.

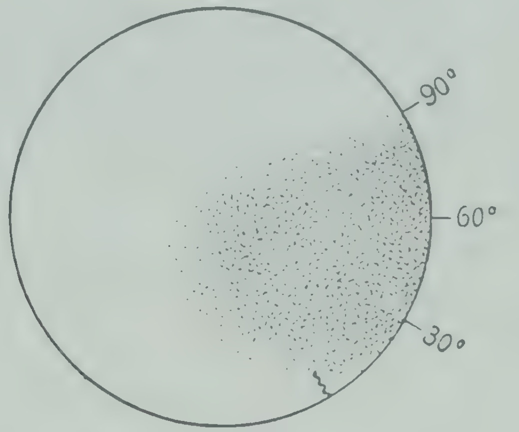

Fig. 63.

ig. 62. The implantation (*Einsteckung*) method of testing for inductive power. *Impl.*, implant.
g. 63. Boundaries of the primary organiser region.

ductive power and in competence, so that the side-plate mesoderm induces less ongly than the dorsal lip and the lateral ectoderm reacts less readily than the ung gastrula ectoderm. But the suggestion was also made that there might an active suppression of the inductive power of the side-plate mesoderm by e rest of the mesoderm, in accordance with the individuation field of the whole bryo. Results closely analogous to these were later obtained by Twitty & denstein in a study of the competence of flank ectoderm to form dorsal fin en transplanted heteroplastically to the dorsal region. Graft of species *A* on st of species *A* gave no induction, but on host of species *B* competence showed self. Conversely a graft from species *B* on host of species *A* showed that some ductive power was present in the latter. These facts teach us that we should ways be prepared to state the context in which we are using words such as ompetence" and "inductive power".

The mesodermal archenteron roof lies, of course, *under* the presumptive neural plate. But the ctice of calling it the "substrate", as some authors occasionally do, cannot be too strongly demned, since this word has a meaning in biochemistry which gives it, in this context, the st misleading implications.

The double layer (invaginated mesoderm and presumptive mesoderm),
the invaginated mesoderm alone, was now known to possess inductive po
What of the presumptive neural plate, and the neural plate itself during
earliest stages of its histological transformation? It had been established tha
process of determination of the presumptive neural plate went on *pari passu*
the invagination of material towards the future anterior end of the embryo
(Fig. 64A). O. Mangold & Spemann now made the discovery that as the de
mination and histological differentiation of the neural plate proceeds, it acquires
power of inducing another neural plate if implanted into the blastocoele ca
of another embryo (Fig. 64B). The term "*Homoiogenetic induction*" was in
duced to describe this effect. Its immediate importance was to draw attentio
the fact that induction cannot be wholly explained as the unit-building activi
of an individuation field. If that were so, an implanted piece of neural tube wo
be expected to call up other structures in its vicinity from the host tissues,
more neural tube.* But these experiments have also a wider significance,
full extent of which has probably not yet been realised (see p. 267). At the sa
time O. Mangold[7] emphasised that irrespective of the age or provenance of
implant, the induced neural tube always appears and develops synchronously w
that of the host (Fig. 64C).

In this same joint paper, Mangold referred to some experiments, then
progress, directed to answer the question how long the neural tissue would reta
its power of induction. These, fully published later (O. Mangold[7]), show
that it could be traced as far as the brain of the free-swimming larva (Fig. 64D
Such observations formed the starting-point for far-reaching work, reference
which it is more logical to introduce at a slightly later stage (p. 172).

From this work, however, it was clearly apparent that the presence of t
stimulus and the presence of the capacity to react to it occupy different tim
spans of the embryo's developmental life. Competence and organiser activity
not arise and disappear at the same time. The former appears only for a relative
short time, the latter seems to begin before and to persist for a very long tir
afterwards. The situation may be summarised in the form of a table (Table 1
constructed partly from the discussion of Spemann ([17], pp. 250–59).

At the blastula stage competence to react to the primary organiser is prese
nowhere. Organiser is present to a slight extent in the region which will becon
presumptive mesoderm and will eventually be invaginated.† At the early gastru
stage, when the blastopore is first visible, competence arises in the presumpti
neural plate‡ and mesoderm;§ this is followed a little later by the appearan

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* Later on, Mangold[11] introduced the terms "*autonomous induction*" for cases where the impl
stimulates tissue to the forming of new structures, "*complementary induction*" for cases wh
the implant takes part itself in what is formed. A discussion of this will be found in Spema
([17], pp. 279 ff.). Homoiogenetic induction by neural tissue is nearly, but not quite alwa
autonomous. In a famous case of Mangold's, a half forebrain was implanted into
blastocoele cavity; it itself formed brain, one eye-cup, and one olfactory pit, but it induced bra
two olfactory pits, two eyes with lenses, four ear-vesicles, one ganglion and one balancer. No
very teleological result (see below, p. 231).

† Bautzmann[1], p. 316.

‡ Spemann & H. Mangold.

§ Lopaschov[3].

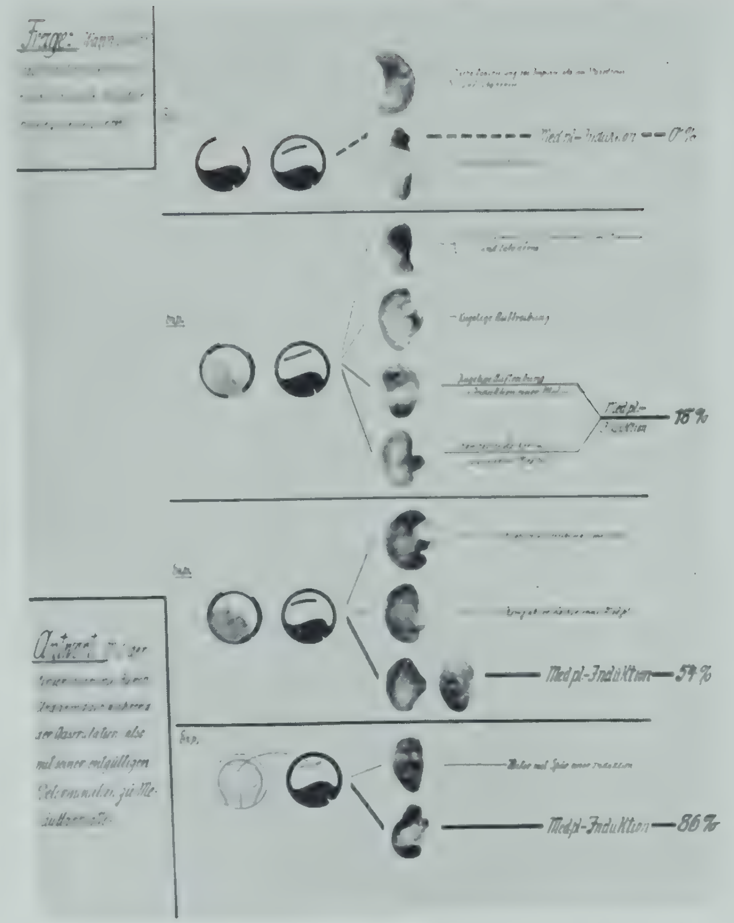
Frage:
Medpl-Induktion —0%
Medpl-Induktion —15%
Medpl-Induktion—54%
Antwort
Medpl-Induktion—86%

Fig. 64A. Determination of the neural plate goes on *pari passu* with the invagination of the underlying mesoderm.

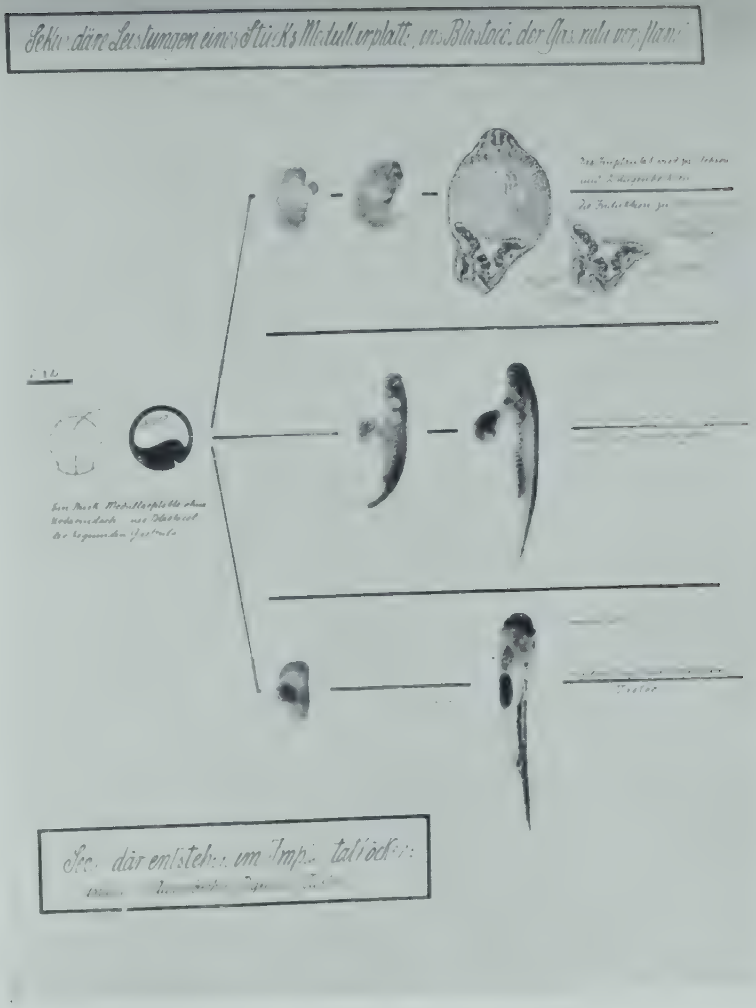

Fig. 64B. Homoiogenetic induction.

Four diagrams of historical interest, prepared by the school of H. Spemann and O. Mangold about 1928, illustrating various aspects of organiser phenomena (continued overleaf).

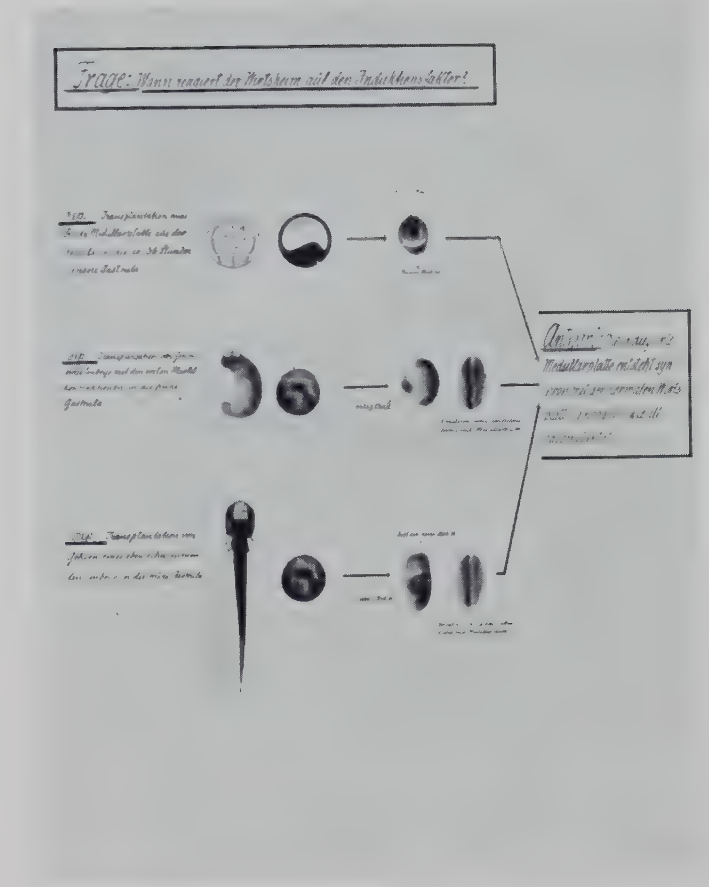
Frage: Wann reagiert der Wirtskeim auf den Induktionsfaktor?
Antwort:
Medullarplatte entsteht syn

Fig. 64C. The induced neural tube appears synchronously with that of the host.

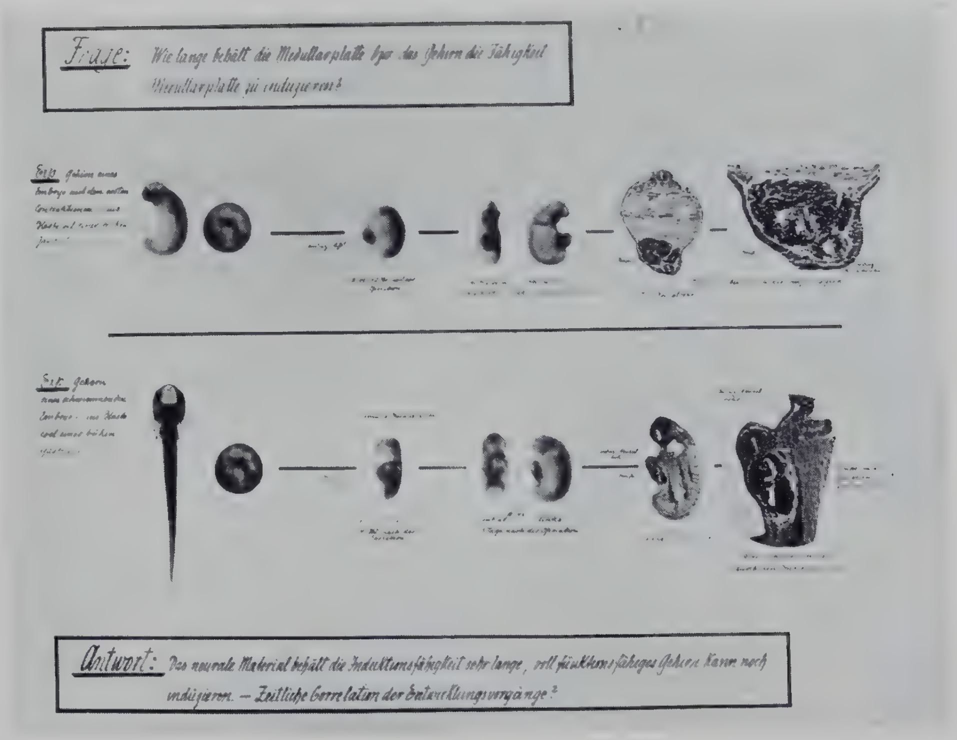
Frage: Wie lange behält die Medullarplatte bzw. das Gehirn die Fähigkeit Medullarplatte zu induzieren?
Antwort: Das neurale Material behält die Induktionsfähigkeit sehr lange, voll funktionsfähiges Gehirn kann noch induzieren. – Zeitliche Correlation der Entwicklungsvorgänge?

Fig. 64D. Inductive power is retained in the nervous tissue at least as far as the brain of the free-swimming larva.

Table 14. *Scheme of appearance and disappearance of competence and organiser action in the amphibian gastrula*

	Presumptive mesoderm		Invaginated mesoderm		Presumptive neural plate		Neural plate		Presumptive epidermis		Epidermis		Yolk-endoderm	
	C	O	C	O	C	O	C	O	C	O	C	O	C	O
Blastula	−	±	·	·	−	−	·	·	−	−	·	·	−	−
Early gastrula (blastopore first visible)	±	+	−	+	±	−	·	·	−	−	·	·	−	−
Middle gastrula	±	+	−	+	+	−	·	·	±	−	·	·	−	−
Late gastrula	·	·	−	+	·	·	−	±	·	·	±	−	−	−
Early neurula (neural folds first visible)	·	·	−	+	·	·	−	+	·	·	+	−	−	−
Middle neurula	·	·	−	+	·	·	−	+	·	·	+	−	−	−
Late neurula	·	·	−	+	·	·	−	+	·	·	−	−	−	−

C = Competence. O = Organiser power.
Presence or absence are indicated by + and − ; a dot indicates that the tissue has disappeared or is not yet formed.

N.B. (1) It will be noted that only in the invaginating mesoderm do competence and organiser power exist side by side. Yet neural tissue does not develop in the archenteron roof. This can only be explained by supposing the competence to be slight, and only manifested when the material is placed in the quite different situation of the presumptive neural area.

(2) Another interesting case is that of the ectoderm which actually covers the just-closed neural tube. It remains ectoderm because its competence is lost just in time. If ectoderm from a younger embryo (gastrula) is substituted for it an additional neural plate is formed (Holtfreter[5]), by homoiogenetic induction (p. 154).

(3) Neural competence exists at much the same intensity all over the presumptive epidermal area and equally throughout the thickness of the cell-layer (Schechtman[6]).

of competence in presumptive epidermis as well.[*] As the mesoderm is invagi it loses its competence but retains, indeed intensifies, its organiser action.[†] A neural plate is determined and later histologically differentiated, it naturally its competence, but at the same time acquires organiser power.[‡] Compe in the presumptive epidermis reaches a maximum during gastrulation, but during neurulation dies away and ceases.[§] Presumptive epidermis or epide of course, never possesses organiser power.[||] Finally, yolk-endoderm n possesses either competence or organiser power.

Of equal interest for the future biochemical analysis were the experimen which Spemann & Geinitz showed that a piece of ectoderm, transplanted the invaginating mesoderm, and subsequently removed from it and placed in blastocoele cavity of another embryo, would manifest acquired organiser capa The significance of this awakening (*Weckung*) or "infection" will later o better appreciated[¶] (p. 205).

The question of the species-specificity of the organiser was early taken Geinitz, only a year after the basic experiment of Spemann & H. Mangold, fo that inductions could readily be obtained by implanting anuran dorsal lip urodele blastocoele cavities (*Bombinator* → *Triton*). Owing to technical d culties, the demonstration that events in the anuran gastrula ran quite para with those in that of the urodele was for a long time delayed, but in the successfully accomplished by Schotté[4]; Vintemberger[1]; and Dalcq & Tung. T converse of Geinitz's experiment was then performed by Spemann & Scho Again, we shall see later on (p. 172) a wide extension of these experiments. anurans the organisation centre occupies a smaller area than in urodeles (Schmi Schechtman[7]).

2·333. Necessity of the primary organiser

From all the work just reviewed it followed inescapably that the organiser cen was involved in the determination of the main axis of the vertebrate embr But how far was it indispensable? We have already seen (p. 103) that a ven half of an embryo, whether originating by constriction of the egg before cleav or by equatorial section of the gastrula, will develop no neural axis. Bautzma found, however, that if a small piece of dorsal blastopore lip was implanted i such a *Bauchstück*, a normal neural axis resulted. Nevertheless, this did prove that the organiser centre was wholly responsible for neural determinat in the normal embryo.

Reasons for thinking that some other factor was necessary arose from distinct lines of work, but both were afterwards proved to have led to fallaci ıclusions. In the first place, Goerttler[1] believed, as the result of his trans- .ntation experiments, that a successful neural induction in competent ventral lateral ectoderm could only be obtained if the implanted organiser was so ented with respect to the host's formative gastrulation movements that its ıvement tendencies were with them and not against them. Altekrüger thought .t successful neural inductions could not be obtained if the isolated organiser s allowed to heal into a ball before implantation. Both these conclusions were :isively proved wrong by Holtfreter[4,5].

---

[*] O. Mangold[2,4,5]. [†] Marx[1]. [‡] Mangold & Spemann.

[§] W. H. Lewis[3]; O. Mangold[5]. [||] Bautzmann[1].

[¶] *Weckung* was subsequently further explored by Raven[4], who allowed a piece of presump ectoderm to remain for only twenty-four hours in the dorsal lip region before being moved a to the ventral region. If it had taken part in the primary invagination it formed notochord muscle and then induced in itself a little neural plate; if it had not, it formed an ectoder thickening only. He was thus able to show that *Weckung* depends on invagination, prob because of the special metabolism connected therewith.

Ihe other line of work had led to results very difficult to understand. Dürken[1] l grafted fragments of the dorsal regions of blastulae or young gastrulae into eye-cups of free-swimming larvae, where they developed well, causing little no reaction on the part of their hosts. The pieces were not dissected out from y well-defined regions, but, nevertheless, neural tissue, notochord, cartilage, ıe, glands, ganglia, etc. were produced, although the material had never lergone the action of the primary organiser in the normal way. The work was ıtinued by Kusche in such a way as to make certain that no organiser had ·n taken for implantation into the eye-cavity. Yet presumptive ectoderm, soderm and even neural plate gave rise to neural tissue, notochord and muscle. out the same time Holtfreter[1] cultivated gastrula isolates in the peritoneal ity of older larvae, with similar results. The phenomena were summarised by ıtzmann[5] under the name of *bedeutungsfremde Selbstdifferenzierung*; i.e. self- 'erentiation in a direction contrary to the prospective significance of the part.* The suspicion had been growing up, however, that the tissues of older animals ght not furnish a perfectly indifferent environment, and so what can be ed the search for a neutral medium began. It was found by Holtfreter[2], who ed that healing of isolates would occur well in a salt solution some twenty s weaker than ordinary mammalian Ringer solution, to which a small amount icarbonate had been added.† After healing, the pieces could remain in the tion or be transferred to sterile tap-water, and would live and differentiate nany weeks.‡

he presence or absence of the organiser centre was now felt in earnest. ›derm, whether presumptive neural plate or presumptive epidermis, isolated ı all contact with the presumptive mesoderm, now developed entirely free

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ıny other transplantation experiments have been made (e.g. rat bone into rat brain (Willis[3]), ın placenta into rabbit eye (Kido[1]), thyroid into eye (May[2]), testis into eye (Bayer & Wense), but, paraphrasing a saying of Claude Bernard's, it is easier to make transplantations than to .ny useful information from them. Nevertheless, the general review of Willis[5] is interesting.

riginally given as NaCl 0·35 %, KCl 0·005 %, $CaCl_2$ 0·01 %, $NaHCO_3$ 0·02 %; $\Delta - 0{\cdot}22^\circ$; ·8–8·2. This composition is not a perfect approximation to that of the normal perivitelline d, as Richards[2] shows; it lacks protein, has too much sodium and chlorine, and is hypertonic

ltures of parts of amphibian embryos have this great advantage over all other forms of tissue re; the cells contain within themselves their natural stores of nutritive materials, and the do not have to be supplied from outside by the more or less hit-and-miss methods of the ımentalist. The contributions of tissue culture in the ordinary sense to the problem of entiation have been reviewed by Bloom. In studying self-differentiation remarkable results 'ometimes be achieved (see e.g. Fell[1,2,3,4]).

from any sign of neural differentiation. Buckled brown masses of cuboidal dermal cells resulted, such as that shown in Fig. 65. On the other hand, if a of blastula or early gastrula was isolated, containing the presumptive meso

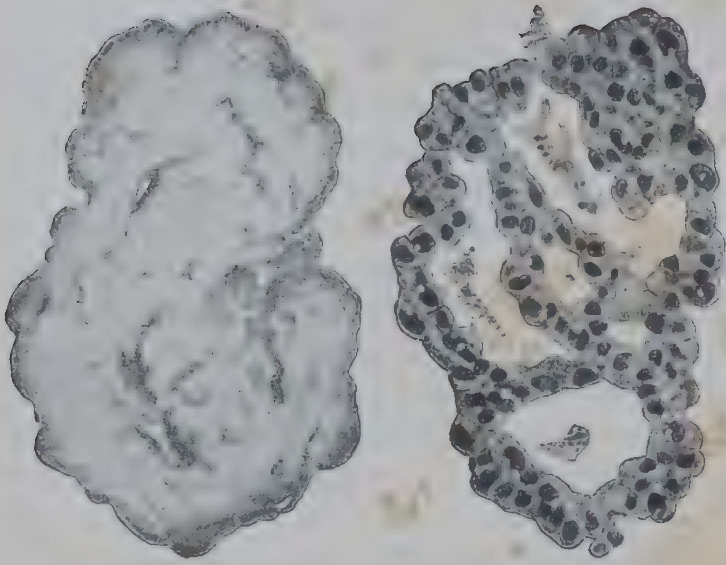

Fig. 65.

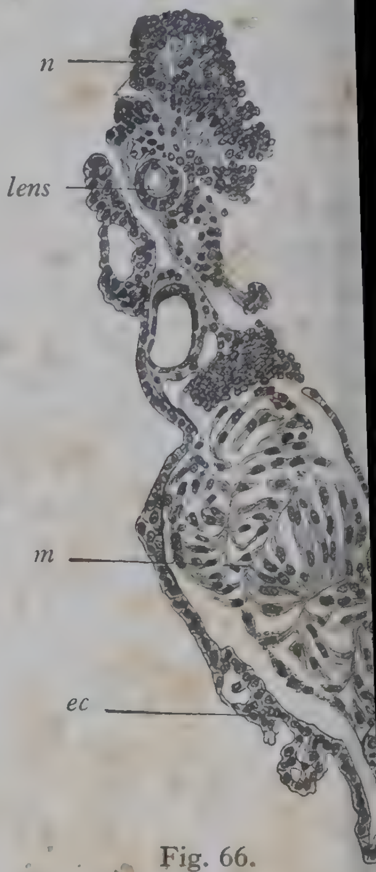

Fig. 66.

Fig. 65. Mass of ectodermal cells formed by explantation of ventral ectoderm in neutral me (external view and cross-section).

Fig. 66. Complex formation of tissues formed by explantation of blastula fragment conta presumptive mesoderm, in neutral medium. *n*, neural cells; *m*, muscle cells; *ec*, ectod

a complicated structure was produced showing all kinds of tissues, muscle, n chord, and neural tissue as well as epidermis. Cross-section of such formati are seen in Figs. 66, 67. The situation may thus be summarised as follows:

	Normal	Implanted *in vivo*	Explanted *in vitro*
Presumptive neural plate	Neural tissue	Neural tissue	Skin
Presumptive epidermis	Skin	Neural tissue	Skin
	(Skin = epidermis)		

Thus, when fully isolated from all organiser action, presumptive neural possessed no more tendency to turn into neural tissue than did presump epidermis. Other experiments by Schechtman[4] have confirmed these result

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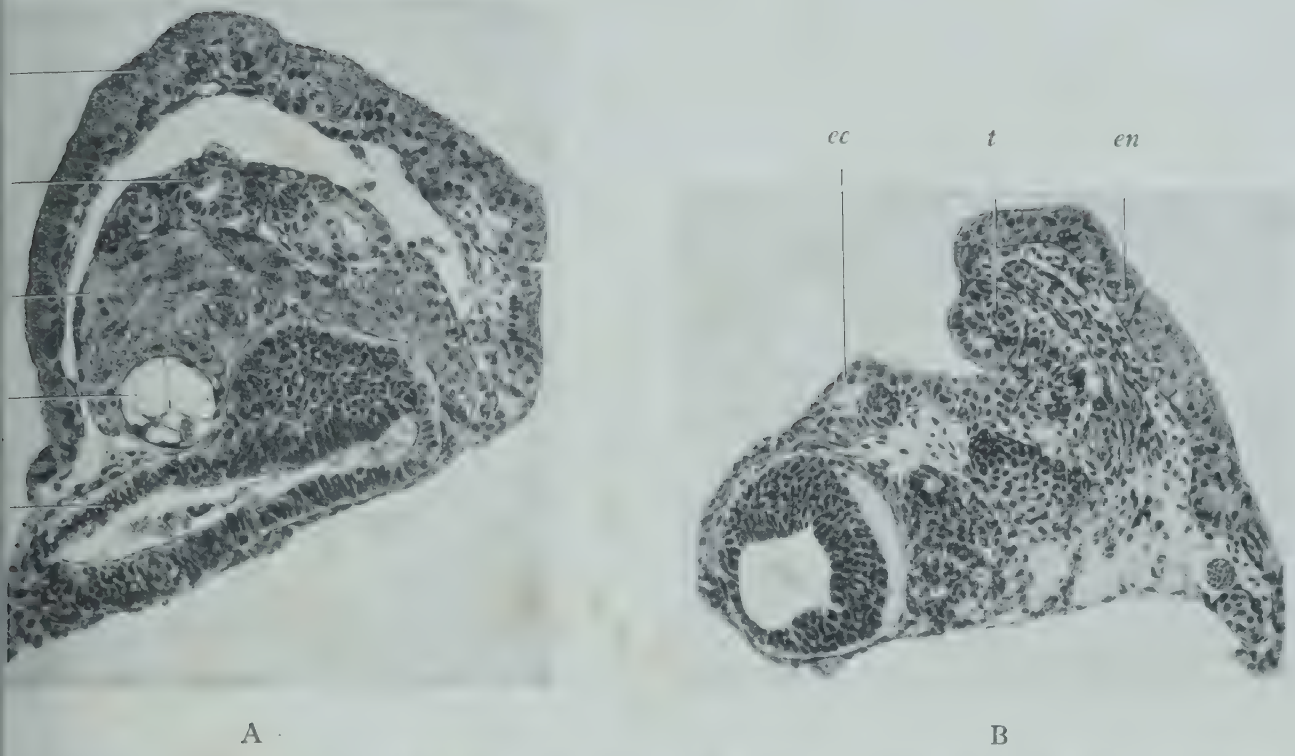
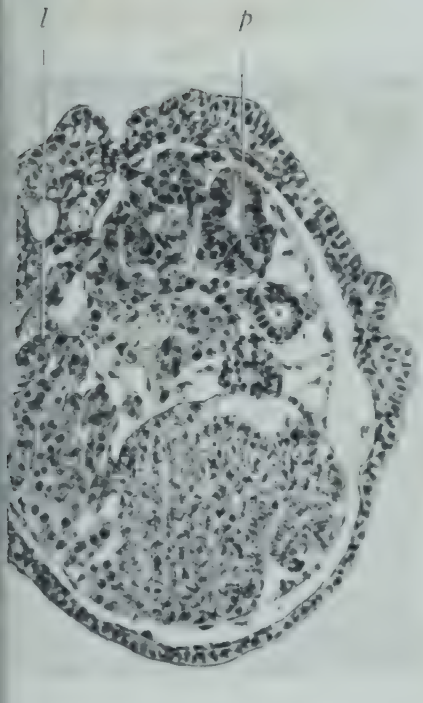
* Cf. the discussion on teratomata below, p. 231.

† The identification of true neural cells in explants may sometimes be a rather delicate m My own experience leads me to concur with the views expressed by Schechtman[4]. "Unde mechanical conditions prevailing in the explant", he said, "some cells become more slende taper towards one end, thus resembling neural cells. But these have no typical neural orientation and disappear as the culture is continued. Typical neural differentiation is ch terised by a compact mass of elongated cells rather than by occasional ones." (See below, p. regarding "palisade" inductions.)



7. Complex formations of tissues arising from piece of early gastrula containing lateral part of organiser. toderm; *en*, endoderm; *k*, kidney tissue; *m*, muscle; *nch*, notochord; *n*, neural tissue; *t*, tooth papilla.



Complex formation of tissues from d endodermal region. *l*, liver; creas.

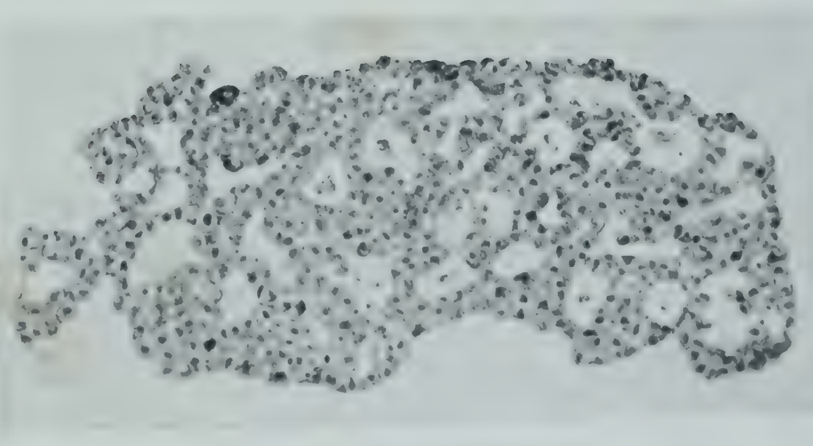
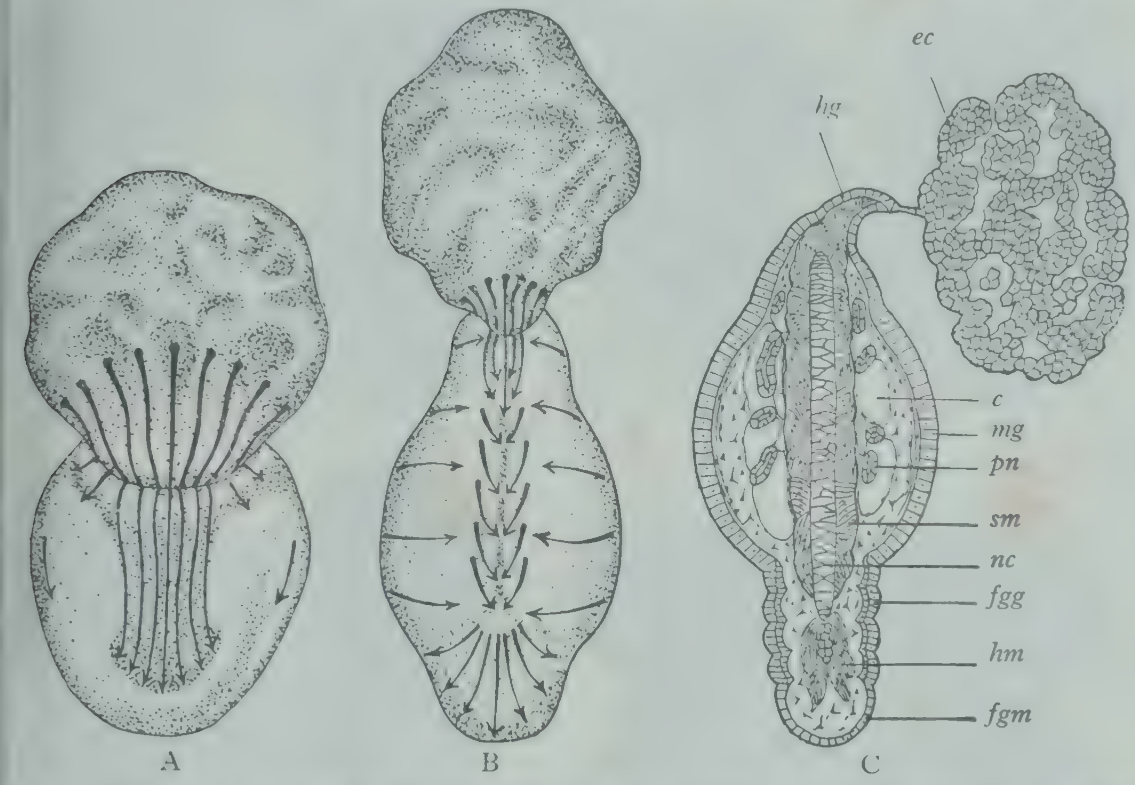


Fig. 70. The ectoderm forsaken by the exoembryo.

The conclusion was in this way reached that the primary organiser was con-
ıned in the tissues of the larval host. As we shall see later (p. 173), the investiga-
ɔn of adult tissues has shown by direct experiment that the primary organiser
ɪes indeed persist in the tissues into adult life. This fact is of much importance
r our understanding of its chemical nature.

Experiments of a quite different type gave the final demonstration that without
e organisation centre of the presumptive mesoderm no neural differentiation
possible. For this purpose advantage was taken of a drastic alteration that may
cur in the formative movements. Instead of turning in at the dorsal lip of the
ıstopore, the presumptive mesoderm may turn outwards, evaginating instead
ınvaginating, so that the material which should have filled up the interior of
ectodermal sphere passes away from it and exerts no influence upon it. Such
-gastrulae, though very undeveloped, were first described by O. Hertwig[1] and
B. Wilson. Holtfreter[8], who discovered a way of producing them in extreme
m at will (cultivation of the blastulae in 0·35 % salt solution*), devoted
ended studies to them and described the result in papers already classical.



69. Diagram of three successive stages in the process of exo-gastrulation. *c*, coelom; *ec*, ectoderm; *fgg*, foregut (gill region); *fgm*, foregut (mouth); *hg*, hindgut; *hm*, head mesoderm; *mg*, midgut; *nc*, notochord; *pn*, pronephros; *sm*, somite muscle.

Ƭhe process is seen in Fig. 69. The whole of the yolk-endoderm is eventually
led out by the evaginating mesoderm, forming an endo-mesodermal mass (the
ːo-embryo") which, by the time a normal neurula ought to have been formed,

---

hey can also be produced by X-radiation (Curtis, Cameron & Mills). An interesting observa-
, which links amphibian exo-gastrulation with that of echinoderms (see pp. 477 ff.), is that
anzi[9], who produces exo-gastrulation of the lamprey (cyclostome) egg by the action of lithium
p. 277).

has considerably stretched itself out. Notochord and mesoderm sink wit exo-embryo, which thus becomes entirely surrounded by endodermal cells will in time give rise to intestinal glands secreting their products to the ex for the whole structure is inside out (Fig. 69 C). Meanwhile, the forsaken dermal ball proceeds to no differentiation of any kind; presumptive epiderm presumptive neural plate alike form a buckled mass of brown cuboidal ecto cells, as shown in Fig. 70 (opp. p. 158). The external appearance of an emb total exo-gastrulation after about a week is seen in Fig. 71. Separation of mesoderm from ectoderm is complete, for the only connection is a thin thr ectoderm which later may spontaneously break. We have thus a self-isolat the germinal layers.

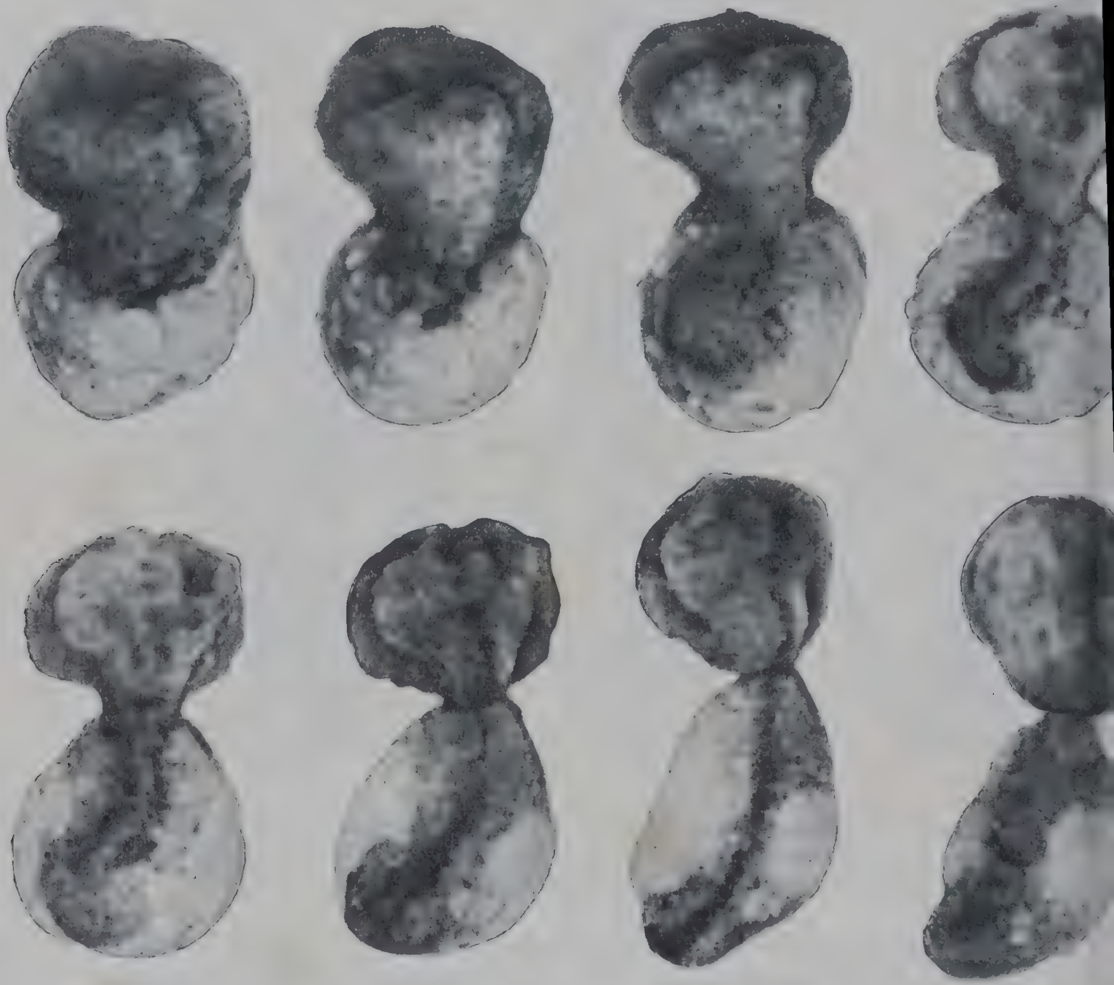


Fig. 71. Successive stages of exo-gastrulation.

In the exo-embryo all kinds of organs develop; pronephros and mesoneph gonads and smooth muscle, a pulsating heart free of blood-cells (for these dev away from it in its new situation), coelomic cavity and connective ti (cf. Figs. 72 and 73). Naturally all these structures are entirely without nerve connections. In the ectodermal part, on the other hand, everythin lacking; there is no neural tube, eye-cup or lens, no mesectoderm, no teeth ear-vesicles, etc.

These results brought final confirmation of the view that the underlying of dorsal ectoderm by the mesoderm is the full, perfect and sufficient cause fo

VIII

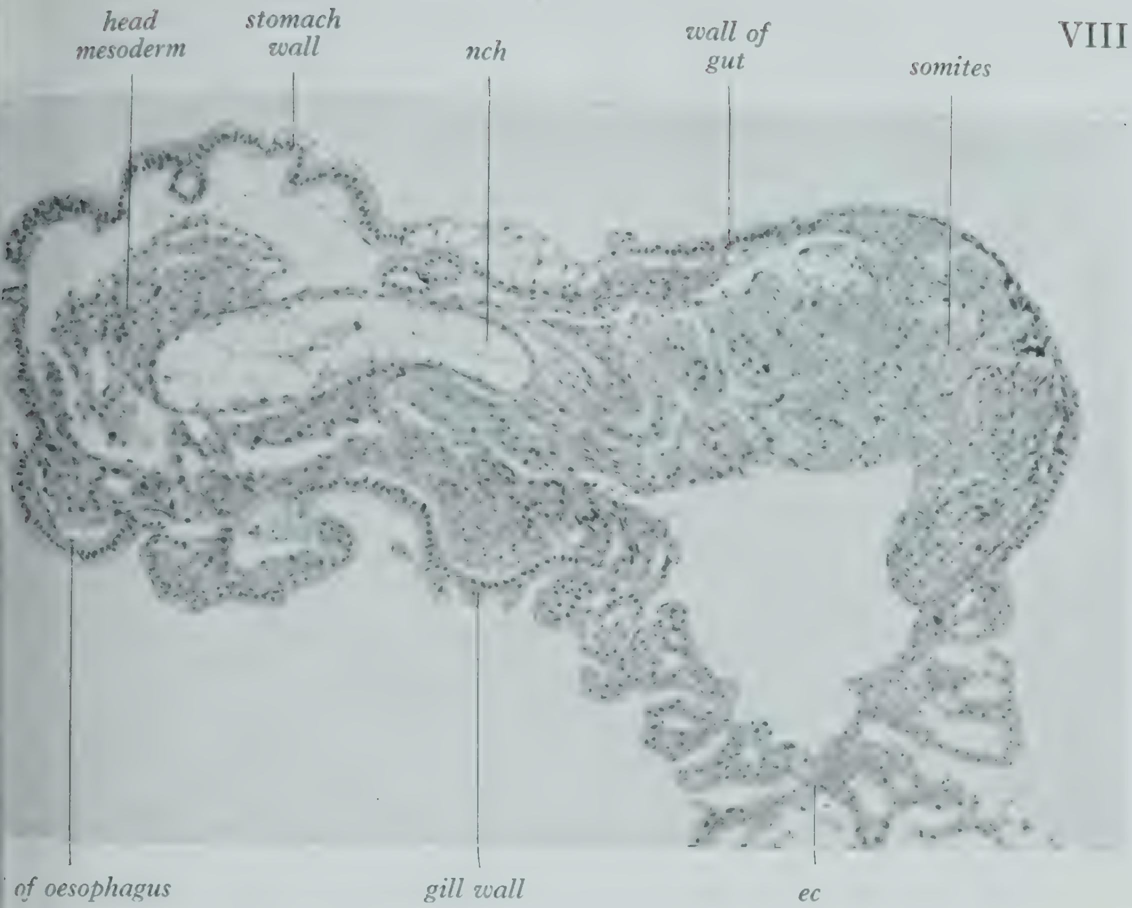

head mesoderm
stomach wall
nch
wall of gut
somites
of oesophagus
gill wall
ec

Fig. 72. Sagittal section through the exoembryo.

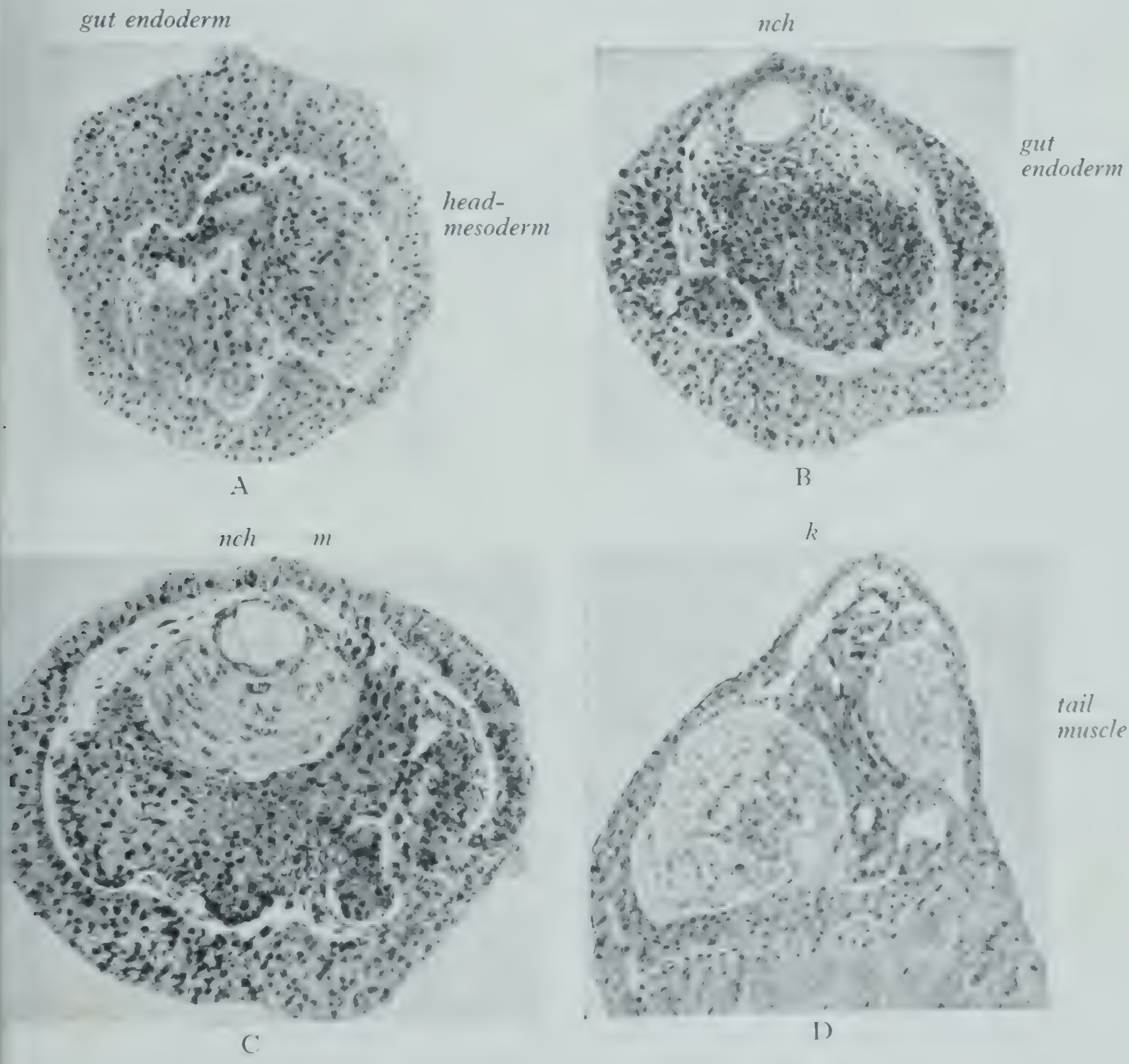

gut endoderm
head-mesoderm
A
nch
gut endoderm
B
nch
m
C
k
tail muscle
D

Fig. 73. Transverse sections through the exoembryo. Conventions as in Fig. 67.

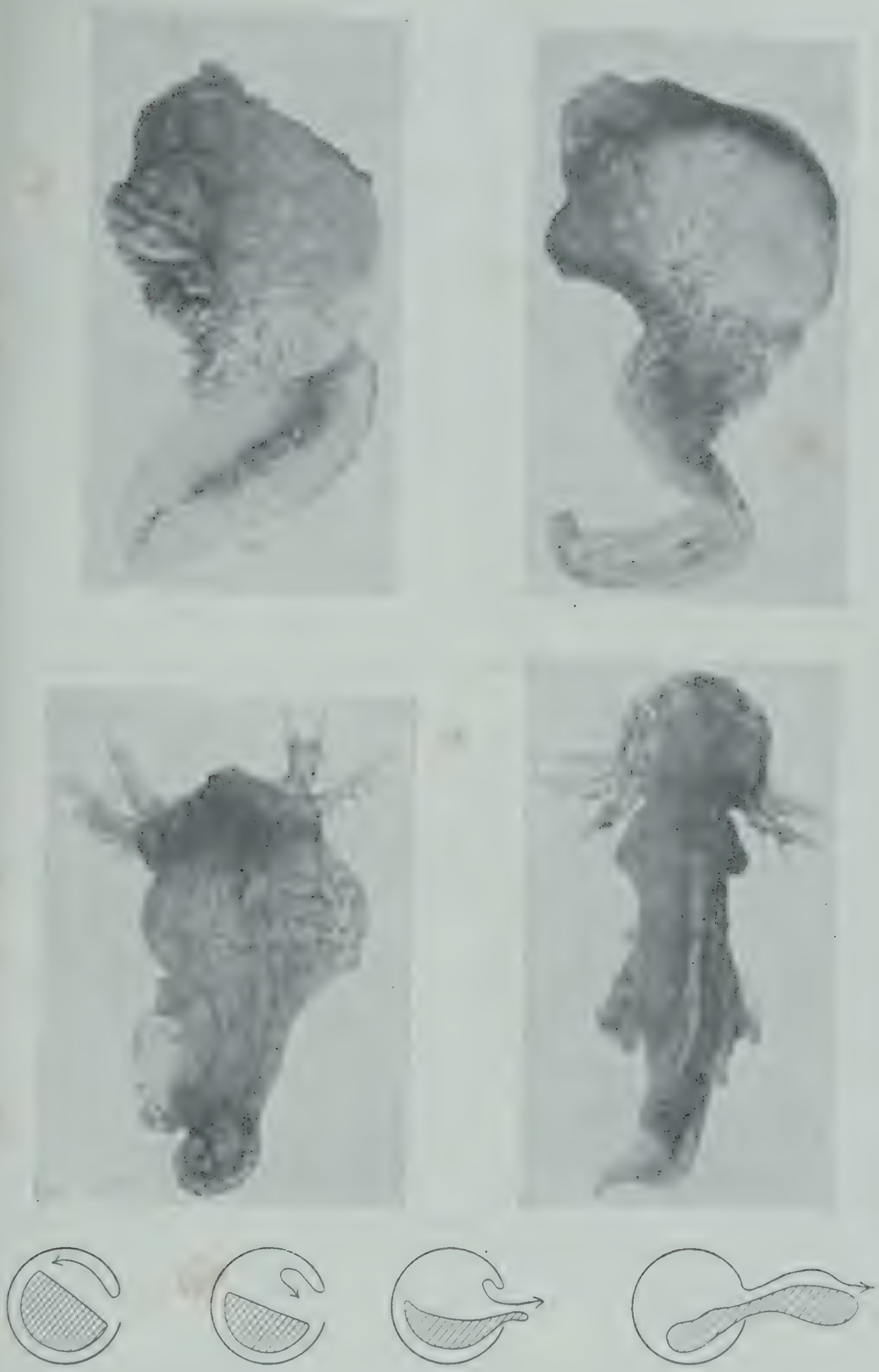
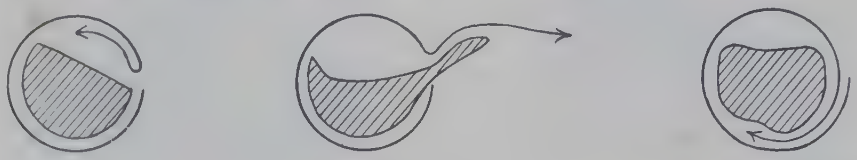

Normal
Partial exo-gastrulation
Total exo-gastrulation

Fig. 74. Stages of partial exo-gastrulation

determination for neural differentiation.* They also negatived a possibility
had at first been canvassed, namely, that the "stream" of determination p
along through the dorsal layer from the lip of the blastopore, rather th
from the roof of the archenteron. If this were the case, inductions would
place in exo-gastrulae, since until a late date there is always a connection be
the endo-mesoderm and the ectoderm. Tests were now made of the capac
the endo-mesoderm to induce and the competence of the ectoderm to re
an implanted organiser. It was found that the endo-mesoderm was quite ca
of inducing neural differentiation in pieces of ectoderm laid upon it anyw
along its length (see p. 273). Conversely, it was found that the forsaken ecto
retained its competence to react to an implanted organiser for a considerable
but that this property was lost some days or weeks before the isolate died.



Normal

Exo-gastrulation
(Holtfreter)

Ventral induction
(Eakin)

Fig. 75. The three logical possibilities of direction of the gastrulation movement.

Such artificial experiments received abundant confirmation from the obse
tion of natural partial exo-gastrulation. Between total exo-gastrulation
normal development all stages can exist. If a small amount of invagination oc
followed by evagination, a small neural plate will be formed which will build
material around it into a quite normal tail. If invagination proceeds fur
before evagination occurs, the whole of the trunk may be fairly normally form
These stages are illustrated in Fig. 74.

When a cell reaches the lip of the blastopore there are three main log
possibilities as to where it may go. One of these happens in normal developm
when it enters the archenteron roof, another in the exo-gastrulation studied
Holtfreter[8]. The third possibility is that it might pass ventralwards and indu
neural axis not in the presumptive neural plate but in the ventral presump
epidermis. This effect was brought about by Eakin[1], who injected a mass of gel
into the blastocoele cavity before invagination had begun. When invagina
should have started, this mass, now hardened, could not be pushed forwards
downwards as the blastocoele cavity usually is, so the mesoderm passed tow
the ventral pole through the yolk-endoderm and induced a neural axis under
yolk, as shown in Fig. 75.

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* It has, however, been claimed that ventral ectoderm may proceed to neural differentia
without the action of the organiser or a chemical equivalent of it. Barth[3] fused together
ectoderm explants, and found that although one alone would not differentiate, neural ma
more or less chaotic, appeared in the fused pieces. For this it was necessary to preserve t
antero-posterior polarity in performing the fusion. The full publication of these results wi
awaited with interest, but it may be noted that the stage from which the pieces are take
admittedly late in gastrulation and therefore very striking proof will have to be required tha
fused mass did not contain small amounts of already determined neural area, which would
homoiogenetic inductions.

2·335. Determination of the endoderm and mesoderm

If we are now prepared to accept the determination of the neural axis by the ,vaginating endo-mesoderm, it must be admitted that much still remains scure. The self-differentiation of endo-mesoderm in exogastrulae, or of isolated doderm (Fig. 68), is remarkable in that no point of origin for this determi- tion can be found. That the dorsal blastopore lip possesses the widest powers self-differentiation cannot be denied, but we are still impelled to search for e organiser of endodermal and mesodermal organs. Practically nothing can t be said upon this subject.

As regards the mesoderm, it has usually been assumed that when it sends out determining influence on the ectoderm it is itself determined. This cannot be , however, for as Adelmann & McLean established, early somites are very erchangeable. Then Yamada[1] confirmed by transplantations between more dely different mesodermal sites that the mesoderm is still very labile at the end gastrulation. In the early neurula, somites were transplanted to the pronephros gion or to the anterior ventral region. In the former they behaved neighbourwise d formed pronephros; in the latter they behaved neither neighbourwise nor fwise but formed pronephros tissue there also. This very surprising result *deutungsfremde Selbstdifferenzierung*) could be inhibited if notochord was nsplanted at the same time; this, as it were, kept them in order, and maintained em as somitic tissue. If a pronephros-inducing substance could be postulated as sting in the anterior ventral region (though normally having nothing to do ere), a suggestion not without plausibility (cf. p. 306), the effect would not be strange. In the later neurula, somite transplants behave selfwise. But the narkable thing is that the mesoderm should still be so labile after having urally determined the ectoderm. As Vogt[5] put it, commenting on Yamada's ılts: "Das Organisationszentrum führt ohne voranzugehen". Earlier work of adley[5] had shown a similar lability of tail-bud mesoderm in the neurula.

ogt[5] distinguishes, on the basis of these results, four stages in determination: There first appears the capacity for self-differentiation to a given structure, ested by isolation into neutral medium; this is present in presumptive noto- rd and mesoderm before invagination. In the second stage, (2), the capacity ssimilate neighbouring tissues by way of regulation arises; this is tested by ect experiments and appears in the early gastrula. (3) The capacity for monious internal regulation (within the germ-layer) appears in the early rula (cf. Yamada), and (4) as this decays, only disharmonious internal ulation remains possible (late neurula). In the nervous system, as well as mesoderm, minor degrees of regulation remain possible for a very long time etwiler[1,2,3,5]; Coghill[1]; R. M. May[3]).

Yamada[3] later investigated the behaviour of somite mesoderm in isolation. ne, *in vitro*, it makes no pronephric tissue. If, however, it is implanted into odermal balls, it will do so; and here again the presence of notochord will vent the transition. But if the ectoderm used for the ball is taken from a iciently young gastrula, the implanted mesoderm will induce a mass of neural ue in it, and this will later act like notochord in maintaining the mesoderm as muscle and preventing the transition to pronephros. Now not only will chordal tissue prevent presumptive muscle from turning into pronephros; i also induce muscle tissue from presumptive pronephros (Yamada[4]). And bet presumptive pronephros and presumptive blood there is apparently a rel similar to that between the muscle and the pronephros, for presumptive p phros will form blood if isolated into ectodermal balls, while presumptive will form pronephros if isolated into ectodermal balls together with notoch tissue. These relationships may be better visualised with the aid of the follo table (Yamada[5]):

	Prospective significance	Result when alone in ectodermal ball	Result when ectodermal ba presence o notochord
Presumptive somite mesoderm	Muscle	Pronephros	Muscle
Presumptive pronephros	Pronephros	Blood-cells	Muscle
Presumptive blood	Blood-cells	Blood-cells	Pronephros

This serial system, obviously of great interest, invites the suggestion that various morphogenetic effects may be due to quantitative variations in amount or activity of a single inductor rather than to the existence of several. weakest concentration would produce blood-cells, a higher concentration wo produce pronephros, and the highest would give rise to muscle. Normally would have to be pictured as radiating from the notochord, but the presump pronephros and blood-forming regions could be regarded as accessory centre its production. The Dalcq-Pasteels theory of "morphogenetic potential" (p. 220), to which Yamada[5] himself inclines, is a somewhat less biochemical way saying the same thing. That something diffusible is at work is strongly indica by the gradations in the effects obtained, the tissue nearest the notochord fr ment, for example, being most fully transformed into well-differentiated mus

Following a parallel line of thought, Balinsky studied[8,10] the lability of en derm. Even when taken from the neurula, yolk-endoderm, if implanted i the blastocoele cavity of a young gastrula, behaves completely neighbourw forming whatever organ of the host it happens to be caught up into. Holtfret exo-gastrulation experiments[8] were to some extent paralleled by the remarka operations of Mangold[13], who completely removed all the yolk-endoderm from young neurula, and cultivated it in salt solution. Such pieces lived on long af all the yolk would have been consumed if they had remained in their norm position, but their self-differentiation was not very striking; they formed o foregut and midgut, no gill clefts, liver or pancreas. Mangold attributed much greater self-differentiation of the exo-gastrulated material to the prese of mesoderm, which again is significant for the argument of this section.

Mangold also examined the results of replacing endoderm in neurulae af inversion of its dorso-ventral or cephalo-caudal axis, or both. The latter seem to be much more fixed than the former.

The problem of endo-mesodermal determination is generally passed over discussions of this subject, and it demands much further work. Is it impossi that there should be appropriate substances present in the invaginated evaginated) material, analogous to the primary evocator?

2·34. Biochemistry of the primary organiser

The analysis of the biochemical properties of the primary organisation centre gan, as all such analyses must, in the enquiry whether or not the integrity of e living cells responsible, in this case the dorsal lip of the blastopore, was quisite for the inductive effect. At the conclusion of their paper, Spemann & . Mangold had written "Aus diese Überlegungen ergibt sich das Experiment, ne etwaige Struktur des Organisators zu rstören und zu prüfen, ob er dann immer och determinierend wirken kann". Less than n years later this question was settled affirma- vely.

Marx[2] was the first to show that normal spiratory metabolism at least could be ex- uded, since induction would still occur after e narcotisation of the dorsal lip of the astopore with trichlorbutyl alcohol and its nsplantation to the blastocoele of a host nbryo. But Spemann[14] in 1931 went further en he showed that crushing the cells had tle or no deleterious effect upon their induc- g activity. Some of his material was later orked over by Krämer, and Fig. 76 shows a autiful secondary embryo produced by the plantation of crushed organiser material.

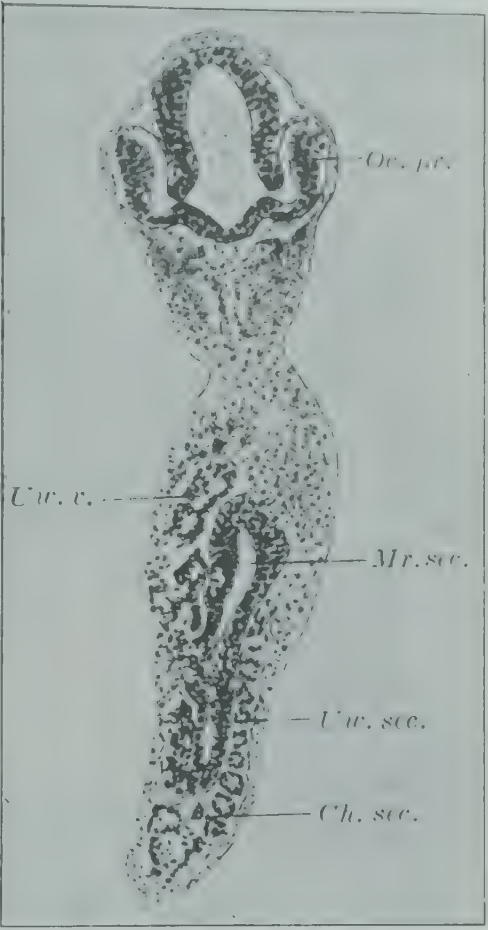


Fig. 76. Induction of secondary embryo by organiser after destruction of its cell-integrity by crushing. *Oc.pr.*, host eye-cup; *Mr.sec.*, induced neural tube; *Uw.sec.*, induced somites: *Ch.sec.*, induced notochord; *Uw.v.*, accessory somite.

In the following year, four workers simul- eously, Bautzmann, Holtfreter, Spemann). Mangold, showed that the organiser was arkably resistant to heat, since it could be led for some time without loss of activity. is definitely excluded the participation of yme effects in the stimulating action. At same time the stability of the primary aniser of the chick to boiling was demon- ted by Waddington[6], and later on the same ved to be true for the teleostean fish in ther's experiments[2]. Holtfreter[6] completed the demonstration in a long and ailed paper which left no doubt that the primary organiser, or, if we confine attention to the neuralisation stimulus, the evocator (see p. 125, and p. 271), s quite stable at 100° C. Coagulated dead pieces of dorsal lip would induce ondary neural axes just as well as they did when living. Holtfreter's experi- ts were of particular interest, for besides the classical technique of implanting material to be tested into the blastocoele cavity of a young gastrula *steckung*), he also used the methods of surrounding the piece of dead material wo layers or sheets of competent ectoderm (*Umhüllung*), and of laying pieces of competent ectoderm upon a flat piece of dead organiser region (*Auflag* Figs. 77, 78, 79 illustrate the consistency and beauty of the results he obta

A digression may be made at this point to emphasise the interest which att to this induction of neural structures, not only in the intact egg, but al spherical masses of ectoderm lying in contact with the source of the evo We have here a new "*Gestalt*", a new arrangement of animal form, br about at will in the plastic embryonic tissue by the experimenter. In no these experiments, however, did any regional differentiation appear, so th effect of the evocator acting from the dead inductor could be described as ducing so many length units of uniform neural tube in the material at its dis

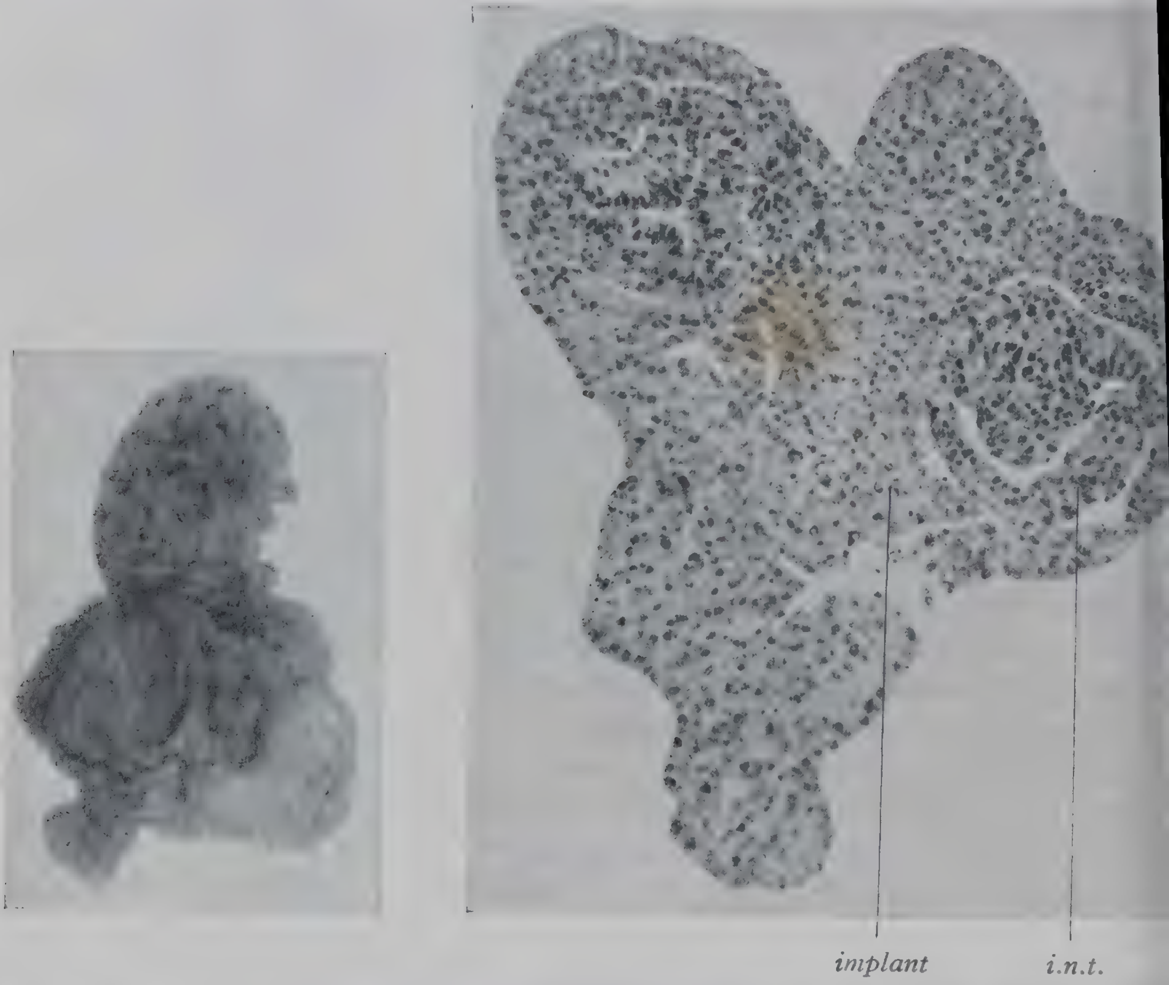


Fig. 78. Induction of two neural tubes by surrounding a piece of boiled organiser with shee competent ectoderm.

It thus became certain that the primary morphogenetic stimulus of devel ment is independent of the life of the cell in just the same way as the adren of the suprarenal gland or the hormone of any other endocrine organ is separa from the life of the glands. Not till later was the converse question asked, nam what degree of integrity must be present in the competent tissue? Here of cou there can be no question of destroying the living cells, since the reaction obviously dependent upon their activity, but normally they are stuck together a definite sheet, pigmented on the outward surface, and probably covered w a cuticle which makes them more permeable from within than from witho

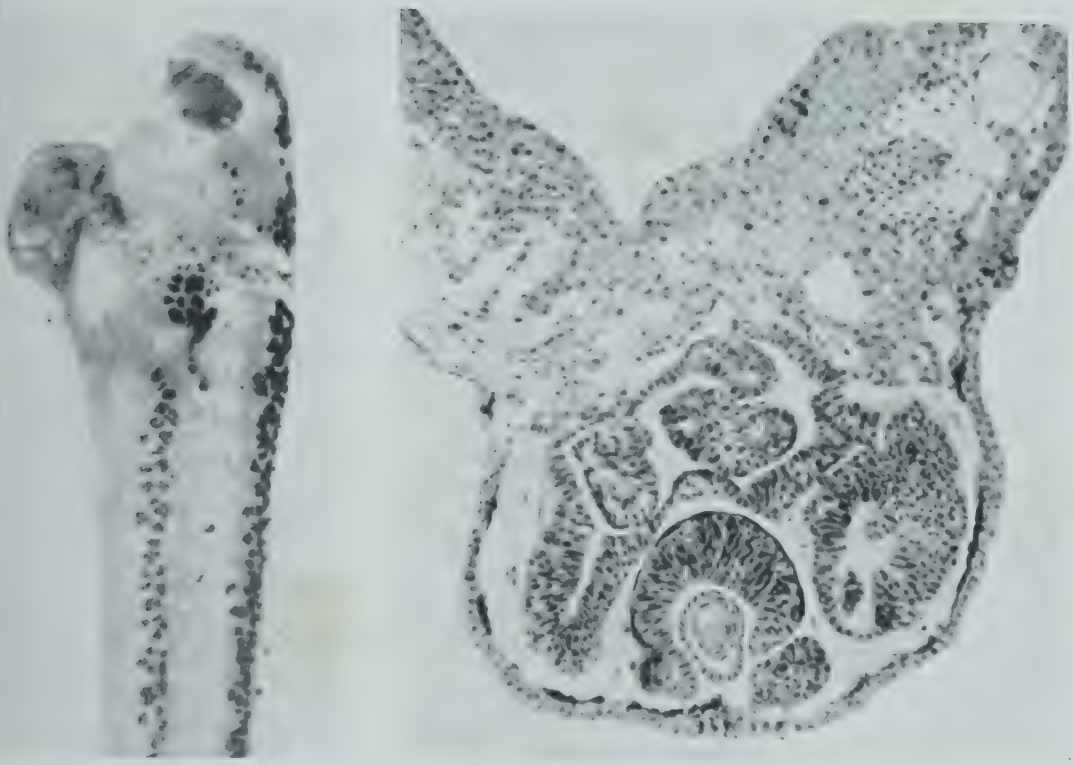


Fig. 77. Induction of secondary brain, eye-cup, etc. by implantation of boiled organiser.

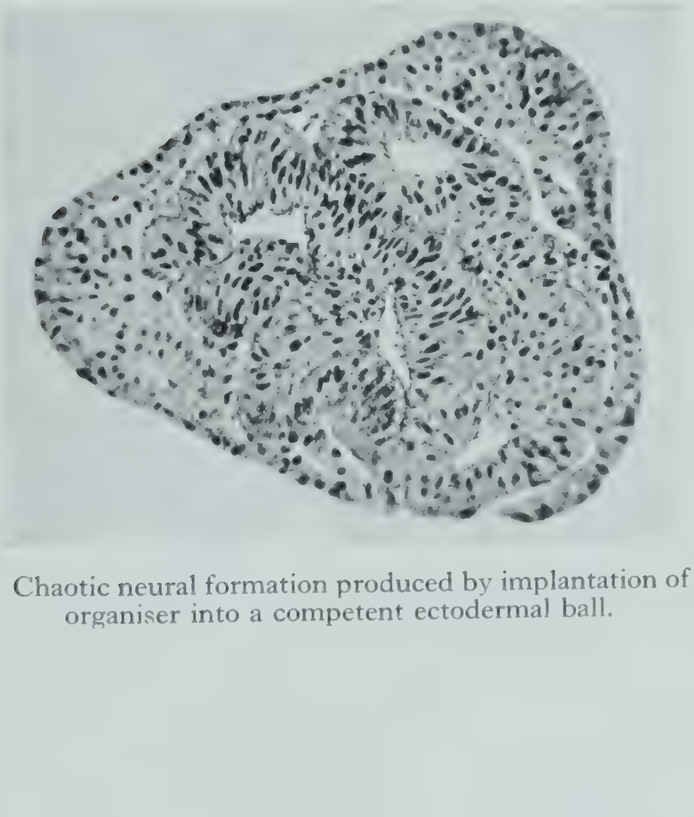
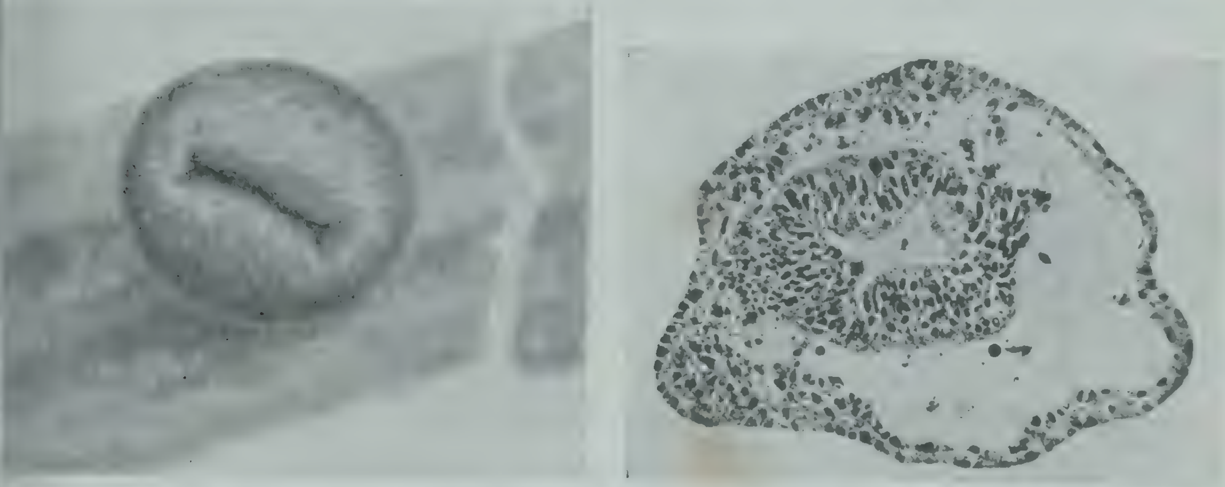


Fig. 80. Chaotic neural formation produced by implantation of boiled organiser into a competent ectodermal ball.

ould induction still occur if these normal relations were interfered with? The iestion received an affirmative answer in the work of Umanski[2], who succeeded breaking up competent ectoderm into its constituent cells with a hair-loop thout injuring more than a few of them. Into the mass so formed, he implanted piece of living dorsal lip, with the result that in many cases neural and noto- ordal structures were produced. From his description, the histological changes ere more marked and normal than the morphological ones. This is perhaps an dication that the immediate effect of the evocator is upon the cell structure, oducing a histological change, and that the morphological changes then follow. A somewhat similar dissociation between the histological and the morpho- gical or "organogenetic" differentiation was met with by Holtfreter[6] in the periments where explants, usually healed into more or less spherical form,*



· 79. Induction of neural tube in a ball of competent ectoderm laid on a piece of boiled organiser.

e laid upon a substratum of dead tissue, or received implants of it. "The logical, tissue-building process", he said, "is more frequently and more cally carried through, than the morphological or organ-building one." Very n brain-like masses of neural cells would be produced, but though they might ound cavities of various shapes, no neural plates or tubes of typical cross- ion were to be seen. A typical case of this rather chaotic neural-tube formation hown in Fig. 80 (opp. p. 166). We shall later return from time to time to this ociability of histogenesis and morphogenesis, especially in the discussion evocation and individuation, and the experiments designed to define the erence between them (Section 2·391). In order to disarm the criticism that difference between the structures produced by dead inductors in explanted ates of competent ectoderm, and those produced by living inductors in the ct embryo, might be due to the reacting tissue and not to the conditions of

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olations of pieces of ventral ectoderm of the gastrula, which then heal themselves into balls into which implants of all kinds may be placed, have proved of the utmost value (see below, 78 and 280). It is interesting that nature seems sometimes to perform similar experiments. land-Sutton (*Tumours*, etc., p. 618) describes rare ovarian dermoid cysts containing thousands pithelial balls" (see below, pp. 232 ff.). The original papers on such cases, however, such as of Bonney, give rather the impression that the balls are of dead cells, sweat, and hair, and do ontain any true epidermis.

the stimulus, Holtfreter made control experiments in which living do pieces were implanted into isolated ectodermal balls. The results wer definite; perfect neural tubes of considerable length were produced t with tails, sense organs, somites, etc.

Formative movements of the ectodermal isolates were observed by Hol who summarised them in the way shown in the accompanying diagram (F A short time after the ball was placed on the underlying dead tissue, it layer of cells began to buckle on the upper surface, simulating the neural At the same time the surface began to be covered with cells which came the lower pole of the isolate and wandered round the outside; these eve formed the "epidermis", while the others formed the neural tissue. In cases, these cells issuing from the lower pole spread out on the supporting s and later, drawing themselves together, rounded up in the form of a dau

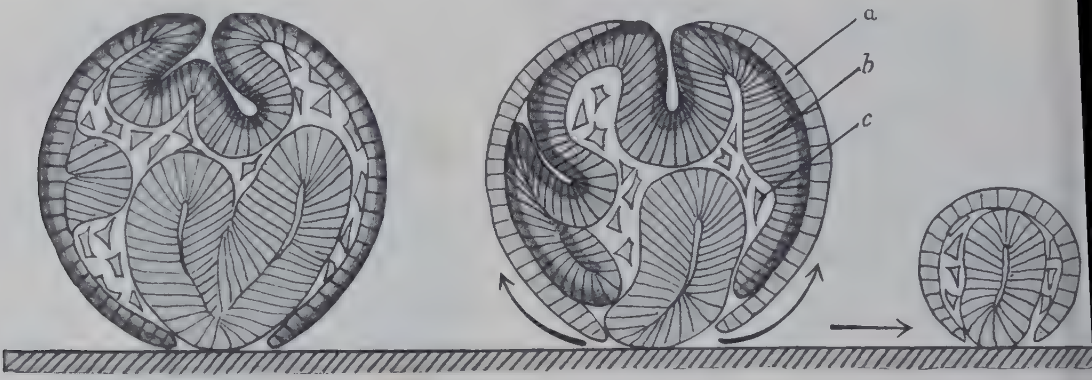

Fig. 81. Formative movements in isolated ectodermal balls resting on dead organiser. *a*, f internal cells; *b*, former outer surface; *c*, mesoderm.

isolate. Holtfreter gave some illustrations of these in section. They contai small tubular or globular mass of clearly neural tissue, surrounded by an dermal "skin".

Observations were also made on the time necessary for inductive effec appear. A twenty-four hour contact between isolate and dead tissue seemed the minimum time after which induction would follow. It must be rememb that the whole technique required strictly sterile conditions, so that the b inductor was not in the slightest state of decay. Induction was prevented ball isolate was allowed to rest on a piece of vitelline membrane, not direct the boiled tissue.

So far, events had given every promise of a fairly straightforward sta affairs. But Holtfreter[6,11] went on to make the remarkable discovery that pa the egg which do not possess inductive power in the living condition, ac it after being boiled. The ventral presumptive epidermis of the gastrula, o ventral epidermis of the neurula, could thus, once dead, call forth as s inductions as had previously only been obtainable by living organisers. the fat, white, yolky cells of the endoderm would actively induce after killed by boiling, and so would the fertilised egg and the egg not yet shed the ovary (see Figs. 84, 85; opp. p. 172).

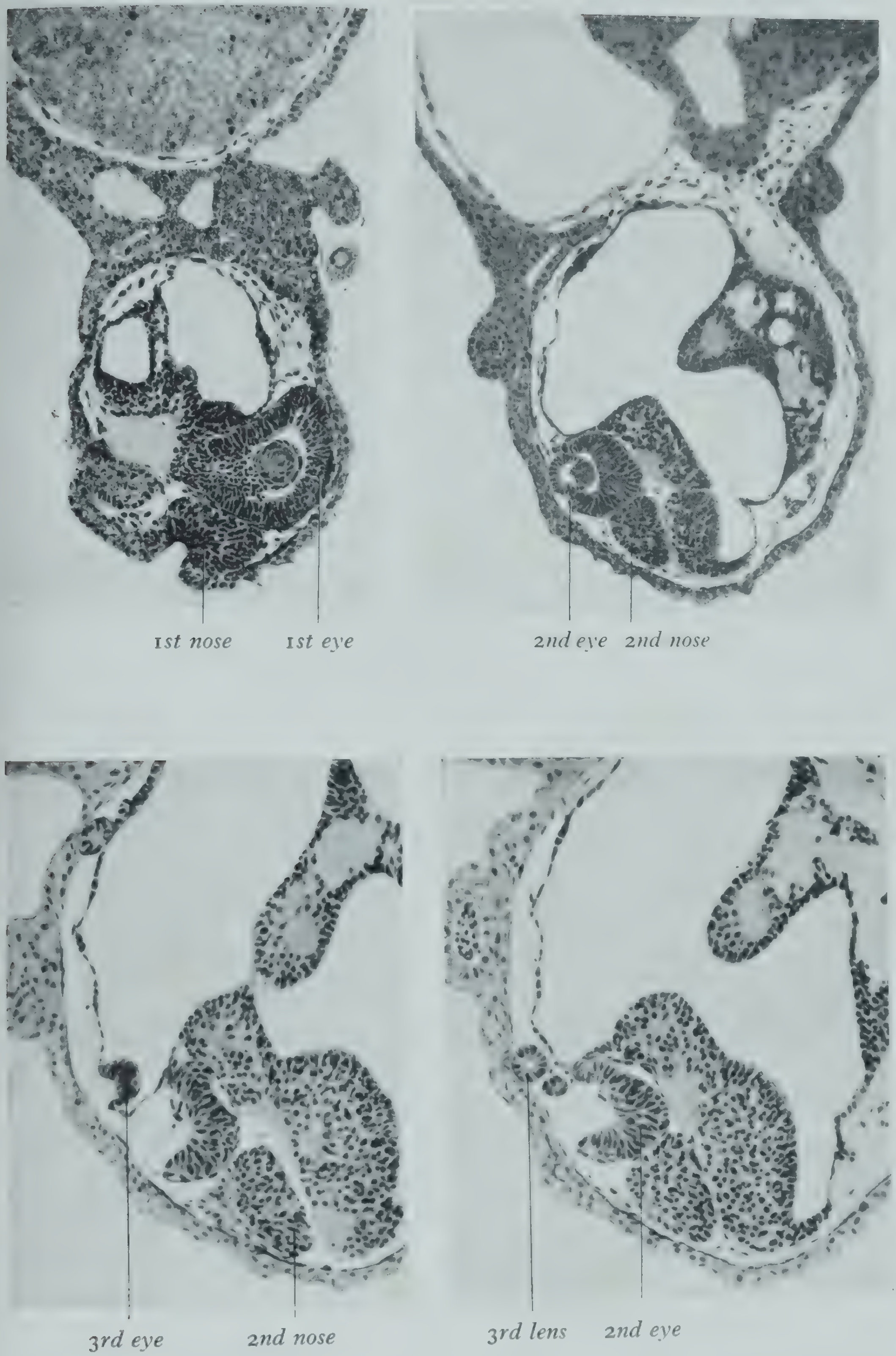
1st nose
1st eye
2nd eye
2nd nose
3rd eye
2nd nose
3rd lens
2nd eye

Fig. 82. Induction of chaotically arranged secondary embryo by implantation of boiled organiser into the blastocoele cavity.

From this extraordinary fact it was at once evident that in normal development e evocator exists all over the egg, but in a *masked* or *inactivated* state.* Only the dorsal lip of the blastopore is it normally set free, and only in the invaginating of of the archenteron does it exercise its profound effect upon the competent toderm (the presumptive neural plate) waiting for it above. And straightway e means by which it is set free acquires the greatest theoretical importance, for shows how the most fundamental morphological interactions may be bound up th correctly running metabolic processes. But there is still another point ich must be emphasised. Presumptive ventral epidermis contains the evocator a masked condition, but another name for presumptive ventral epidermis is mpetent ectoderm, and it is precisely such parts of the egg that we are bound use as reactants for the biological test of inductive capacity. The seriousness this fact will become clearer when the progress of the chemical work has been rther described.

In the second of Holtfreter's two great papers on this subject[11] a number of new ints were raised and questions answered. It was noted that the implantation dead organisers into the blastocoele cavity of embryos seemed to lead to very oriented, chaotic, yet well-formed structures. An example is shown in Fig. 82 p. p. 168). Here the posterior part of a neural plate was heated to 90° for a minutes; on implantation a large structure was produced on the host in which re found, besides brain masses, three eyes with lenses and two noses. We shall other striking examples of this in a later section (p. 233). The differences tween inductors and reactors may then be summarised as follows:

Inductor	Reactor	Result
ing organiser	Intact embryo	Perfectly formed secondary embryos, showing lateral symmetry and organs correctly formed and placed
d organiser	Intact embryo	Organs well formed, but often in excessive number, and chaotically placed. No marshalling of them into a secondary embryo
ng organiser	Ectodermal isolate	Perfect histogenesis, organs small but usually placed in fairly normal relationships
l organiser	Ectodermal isolate	Perfect histogenesis, but little or no organ-formation. Neural cells arranged round irregularly branching cavities

this can hardly be interpreted save in terms of evocation and individuation below, Section 2·391).

nother strange fact was that in some cases a lens alone might be formed from ectoderm, in the complete absence of any eye-cup. This is another phenomenon ch we shall meet with again (p. 299). In the present context, it is noteworthy t it was especially likely to happen if the inductor had been boiled for a long

n alternative interpretation was advanced shortly after Holtfreter's discovery by Spemann, her & Wehmeier, who suggested the existence of an inhibitory substance (*Hemmungstoff*) hose parts of the embryo which do not induce in the living state. They believed that by tment with organic solvents, etc. this inhibitory substance could be extracted. There seems eed, however, to have recourse to the hypothesis of an inhibitory substance when the much ler one of combination of an active principle with a protein, for example, in an inactive plex, is at hand. In a sense, of course, the protein would be the inhibitory substance. But ypothesis, in the sense in which it was advanced by Spemann *et al.*, regarding the inhibitory tance as one of low molecular weight, has not proved fruitful and is to-day generally aban- ed.

time, thus suggesting that the lens-inducing substance might be more stal heat than the primary evocator (see Fig. 83).

Prolonged boiling, however, does not destroy the inducing action, th Holtfreter[11] found slight indications that after 2 hr. boiling its activity was less More important was the fact that if the tissue were heated to 135° the ef were very greatly diminished, while a temperature of 150° completely aboli them. Ashing naturally gave the same result.* As regards the effect of va chemical agents on the activity of inductors, Bautzmann *et al.* had already repo that a stay in 96 % alcohol for 3 min. had not affected the dorsal lip. This confirmed by Holtfreter, who found that the activity had not been lost by pi conserved in strong alcohol for many months, nor even by pieces subseque treated with xylol and paraffin wax. Activity was unaffected by concentr hydrochloric acid or by ether.

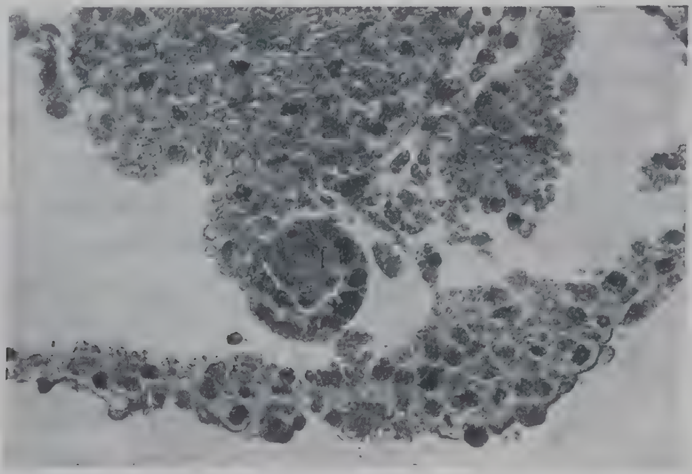

Fig. 83. Induction of a lens in the absence of any eye-cup after implantation of boiled organiser into the blastocoele cavity.

Holtfreter's new contribution[11] was to show not only that the activity of a liv inductor was not affected by these treatments, but that activity was conferred them upon a living non-inductor. Thus presumptive epidermis or yolk-endode after treatment with organic solvents such as alcohol, ether or xylol, would ind just as well as if they had been boiled, and the same result was obtained by freez followed by drying. This implies that any treatment which denatures the prote of a non-inductor will cause inducing power to appear, i.e. will liberate evocator from its inactive combination.

It is often not realised how this fundamental discovery of Holtfreter's clari facts of a morphological order which would otherwise be almost inexplica Thus Töndury[1] replaced dorsal lip material (presumptive chorda-mesode with presumptive neural plate ectoderm; the new dorsal lip invaginated v well and duly induced a normal neural axis in the overlying tissue. If we did know that the primary evocator has to be liberated from its inactive complex, that this is somehow or other associated physiologically with the inrolling at dorsal blastopore lip, the possible transposition of these two regions would ery hard to understand. Conversely, the uninvaginated presumptive mesoderm ould, when transplanted, make neural tube and ectoderm, since its evocator had ever been properly set free.

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* When I reported this fact to Otto Warburg in his laboratory at Berlin-Dahlem in 193 remarked, with a characteristic twinkle in his eye, "Dann ist es nicht Eisen".

In analysing his results from the morphological point of view Holtfreter[11] oted the significant fact that regional specificity was not conserved by dead ductors. Consideration of this, however, must be postponed till a later section . 277). Another striking result was that there was no correlation whatever tween the kind of treatment which the killed organisers had received and the orphological structures which they subsequently induced. Whatever the treatent, brains, noses, eyes, ear-vesicles, lenses, balancers, and mesoderm were to found in each series. When the size of the inductions was taken into consideraon, it was found that the activity of "activated" non-inductors was rather less an normal inductors:

	Average size of induction (Holtfreter units)
Dorsal lip region (killed)	1·43
Presumptive epidermis (ventral ectoderm; killed; activated)	1·06
Yolk-endoderm (killed; activated)	0·67

is might authorise the view that there is actually rather more of the evocator esent in the region where it is normally liberated or activated than in those gions where it is not.

It was said above that the unfertilised ovarian egg was found by Holtfreter[7] to active as an inductor if boiled. Since the nucleus is of so relatively large a size the ovarian egg, this invited a test of relative inducing activity as between cleus and cytoplasm. Carried out later by Waddington[19], it showed that the cleus possesses considerably more inductive power than the cytoplasm (see gs. 84 and 85).

	% inductions	Av. vol. $\mu l . 10^{-4}$
Nucleus	57	92
Cytoplasm	28	14

n the meantime, a number of investigators were pushing Geinitz's original overy of the absence of species-specificity of the primary evocator to much ater lengths. The successful heteroplastic inductions of Waddington & midt on chick and duck were but the counterpart of the older urodele-anuran splantations, but Waddington[7] was able to induce a secondary embryo in the it blastocyst by a chick organiser, and a secondary chick embryo by a rabbit aniser[11]. In amphibian embryos inductions were produced by chick primitive ak* (Hatt[1]), by teleostean fish organisers† (Oppenheimer[4]), and by lamprey aniser‡ (Bytinski-Salz[4]). The converse implantation of amphibian tissue under npetent fish embryo tissue was also successful§ (Luther[2]). Inductions in phibian ventral ectoderm were brought about by the implantation of a enerating larval limb bud (Umanski[1]‖) and of pieces of rat carcinoma and cken sarcoma (Woerdeman & Hampe). Krüger[1] alone failed with lens lantations from older embryos, but this was later successful in the hands of tfreter[12].

allus domesticus in Triton taeniatus. † *Danio rerio* in *Triturus torosus.*

mpetra planeri in Molge vulgaris. § *Triton taeniatus* in *Salmo fario.*

got better results from well-developed buds than from the youngest blastemas.

The ground was therefore in a sense prepared (though this did not less
surprise of it) for the discovery of Holtfreter[7, 12] that the evocator was not co
to the developing embryo, but that all adult tissues of all phyla indiscrimi
would, if implanted into the blastocoele cavity, induce a secondary em
A typical result is shown in Fig. 86, where a secondary brain has been in
in the ectoderm and a secondary gut in the endoderm by the presence of a
of boiled mouse heart. The following species were tested: two worms, two mol
three insects, a crustacean, two fishes, three amphibia, a reptile, two birds
mammals, and man. From Table 15 emerges the conclusion that althou
the tissues tested were active, those of vertebrates were in general more so,
quantitatively and qualitatively, than those of invertebrates. A typical inducti
invertebrate tissue is shown in Fig. 87, where a small brain has been induce
the pupal lymph of the hawkmoth *Deilephila euphorbiae*. About the same tim
inducing activity of adult newt tissues was established by Needham, Waddin
& Needham. Later G. A. Schmidt[12] published further experiments of the
kind, using anuran instead of urodele gastrulae. Holtfreter's series was su
quently completed by Waddington & Wolsky, who tested pieces of the coelent
Hydra viridis, never the possessor of any neural axis itself, as inductors i
amphibian blastocoele, with positive, though rather weak, effects.*

Especially significant was Holtfreter's finding[12] that not all these adult tis
required boiling or other denaturation of their proteins to manifest their activ
But like the organiser region of the embryo, they did not lose much of their acti
even on prolonged boiling. This point was later more precisely investigate
Chuang, who found that if mouse kidney was kept at 100° C. for 2 hr. practi
all its activity was lost. With newt liver especially instructive results were
tained. As the accompanying figures show:

Percentage inductions

	Ectodermal					Mesodermal			
Newt liver	Brain	Nasal placode	Eye	Balancer	Ear-vesicle	Tail	Muscle	Noto-chord	Pro ph
Fresh	73·2	13·6	9·6	9·0	46·4	47·0	31·3	13·6	1
Boiled 2 sec.	34·6	40·1	13·3	28·3	5·5	0	0	0	
Boiled 5 min.	68·4	68·4	49·3	26·0	16·4	0	0	0	
Boiled 15 min.	38·1	53·4	23·6	31·2	24·4	0	0	0	

coagulation of the proteins immediately reduces all mesodermal induction
quencies to zero. The substance or substances responsible must therefore

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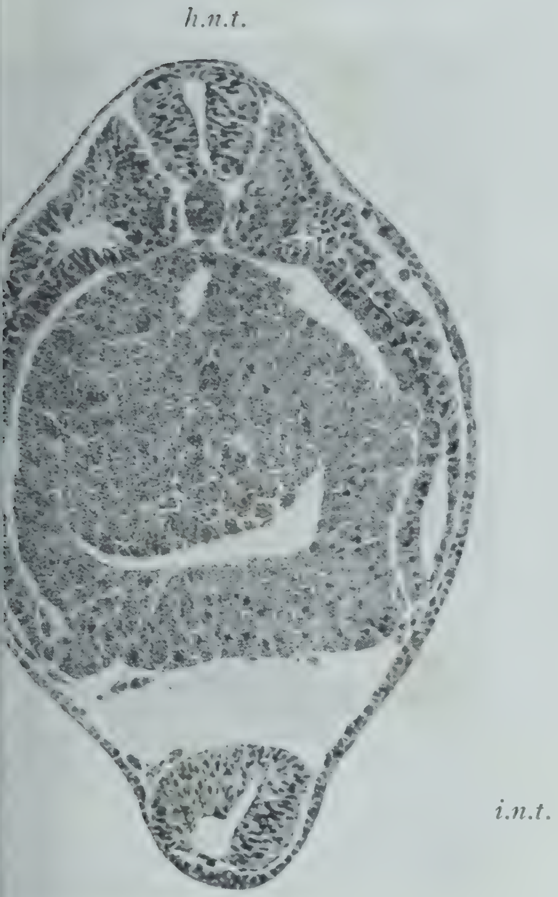
* This would mean that a new step in evolution may involve the introduction not of a new st
latory substance but rather of a new reaction to a substance already present. Many instance
now known in which substances having a more or less specific biological effect are found t
remarkably widespread, occurring even in organisms where their effects cannot be manife
(e.g. oestrogenic substances in plants; Skarżynski[1]). D. L. Thomson had already followed
line of thought when he called attention to such facts as these: (1) adrenalin is present in ann
but does nothing there; (2) the posterior pituitary substance causing uterine contractions is f
in selachian fishes; (3) there is oestrone in insects though they do not react to it; and conver
(4) mammalian pituitaries contain the amphibian melanophore-expanding substance, and
the avian prolactin (see p. 76).

† Perhaps it is a failure to realise this which makes Barth & Graff reluctant to admit
the primary evocator is contained in adult tissues and also, in masked form, in those parts o
embryo where its activity is not normally manifested.



nduction of secondary neural tube by im-
n of boiled nucleus from an ovarian egg.

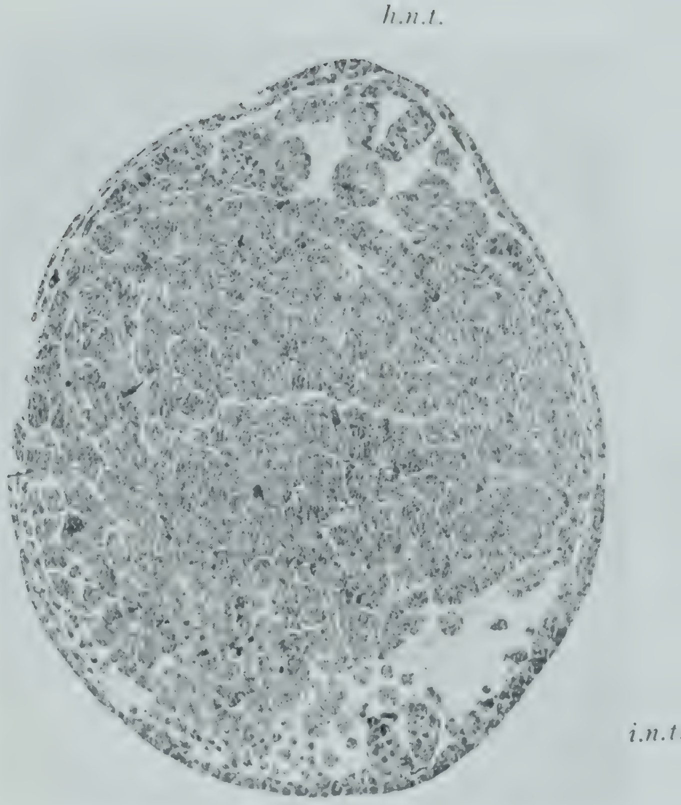
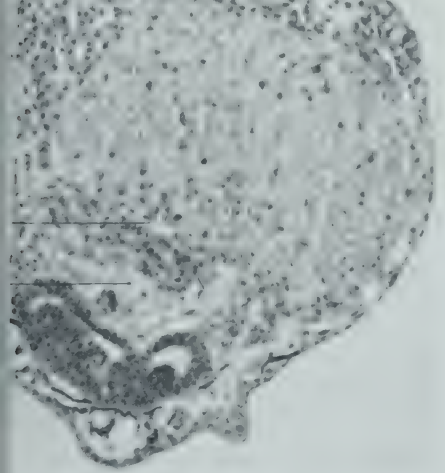


Fig. 85. Weak induction performed by boiled cytoplasm from an ovarian egg.



. Induction of secondary embryo by
antation of a piece of boiled mouse
tissue into the blastocoele cavity.

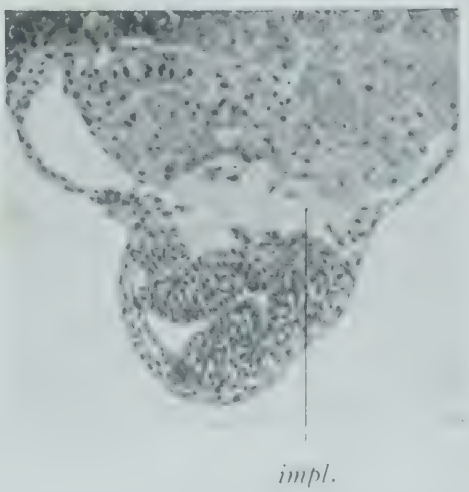


Fig. 87. Induction of secondary brain by implantation of lepidopteran tissue into the blastocoele cavity.

y heat-labile. For ectodermal inductions there are several alternatives; the quency may first fall, then rise and finally fall again (as in neural induction lf), or it may first fall and then continuously rise (as in ear-vesicle induction), it may continuously rise from the beginning (as in balancer induction). The nplest explanation of these differences is that the frequencies are in every case resultant of two opposing processes, the liberation of the active substances m inactive protein-bound precursor complexes, and the inactivation of the stances themselves by heat. We shall return later to Chuang's important k in connection with individuation and second-grade inductors (p. 279), in ich the study of temperature effects was continued.

Table 15. *Distribution of the primary evocator*

	Weak inductions; e.g. neuroid thickenings, palisades, etc.	Strong neural inductions; e.g. small neural tubes or cavities	Very strong neural inductions; e.g. big brains
nmals			
Homo sapiens	—	—	*T*, *K*, *L*, *B*, *t*
Mus musculus	*b*, *F*	*l*, *npl*	*K*, *L*, *B*, *A*, *H*
Bos taurus	—	*L*	*L*, *Me*
s			
Gallus domesticus	—	*npl*	*ee*, *B*
Lanius minor		*T*, *K*, *L*, *S*, *A*, *F*	—
tiles			
Lacerta agilis	—	*K*, *S*	*L*
hibia			
Rana esculenta	—	*M*, *r*	*L*, *Me*
Triton alpestris	*r*	*H*, *Lb*	*B*, *L*, *C*
Salamandra maculosa	*L*, *H*	—	*B*, *r*
es			
Gasterosteus aculeatus	—	—	*H*, *o*, *M*, *L*
ts			
ibellula sp.?	*F*	*N*	—
eilephila euphorbiae	*N*	*pl*	—
anessa urticae	*id*	—	—
iscs			
imnaea stagnalis	*fM*	*L*	—
lanorbis corneus	*fM*	—	—
acea			
aphnia pulex	—	*e*	—
ns			
igula simplicissima	*sob*	—	—
nchytraeus albidus	*M*	—	—
nterates			
ydra viridis	*sob*	—	—

T, thyroid
K, kidney
B, brain
L, liver
t, tongue
H, heart
A, adrenals
r, retina
M, muscle

o, ovarian eggs
e, extract
ee, embryo extract
l, lens
S, testis
F, adipose tissue
N, nerve tissue
b, blood
C, cartilage

Lb, limb-bud
pl, pupal lymph
id, imaginal discs
sob, section of whole body
fM, foot muscle
Me, muscle extract
npl, tumour

hat is the meaning of this remarkably wide distribution of the evocator? en side by side with the facts already described regarding its presence in

masked or inactivated form in all parts of the embryo except the organiser r
the only possible conclusion is that we have to deal with a substance or subs
of extremely wide distribution, powerless in the adult owing to the co
loss of the competence for histological and morphological differentiatio
requiring a kind of "protection" in the egg and early embryo except pr
in that region where the neural axis must arise. The situation may be summ
in the form of four logical possibilities, all of which are realised in fact at dif
parts of the individual and different stages in its history.

	Inducing substance (evocator)	Active inducing substance (evocator)	Competence
Dorsal lip region	+	+	+
Ventral ectoderm	+	−	+
Yolk-endoderm	+	−	−
Adult tissues	+	+	−

Tissues with none of these properties might not be very interesting, but a t
which possessed competence but no evocator would be exceedingly useful
test-object. Unfortunately, no such tissue is known.

In Holtfreter's experiments on ovarian and just fertilised eggs, the mat
was boiled (for technical reasons). This led to the view that the masking com
is not broken down until the appearance of the dorsal lip. But B. Mayer[2] s
that blastomeres from the four-cell stage will, if implanted fresh in a comp
ectodermal ball, induce a neural tube in it. Masking may therefore not b
until some time during the cleavage stages. The full publication of these re
will be awaited with interest.

It is to be noted also how these results of Holtfreter's link up with the
Mangold-Spemann experiment of homoiogenetic induction (p. 154). Ne
plate, once formed, will induce more neural plate in competent ectoderm.
will somites or notochord. It is thus clear that at some stage or other, which
be different for each tissue, all the tissues and organs will take on indu
power, i.e. the inducing substance in them will be liberated from its ina
precursor. The homoiogenetic induction itself was the subject of a pretty ex
ment by Holtfreter[18], in which he showed that mere ageing will not of itself su
to liberate the evocator in presumptive neural plate. Presumptive neural
was isolated in salt solution where, of course, it underwent no further differe
tion. After two days, when control embryos were well-developed neurula
was implanted in the blastocoele of a host. No inductions were ever obta
in this way. Therefore the process of liberation of the active substance mus
connected with the processes of determination and histological differentia
Liver develops from the trunk endoderm, which is inactive to begin with.
at some later stage, the evocator must appear in free form there, since liver ti
is one of the most powerful adult tissues. Muscle, on the other hand, posse
it, like all the mesoderm, from the first.

The structures induced by adult implants are very various. Just as hap
with coagulated embryo organisers, there is a tendency for the productio
excess formations. Thus in Fig. 88 a piece of boiled salamander brain indu

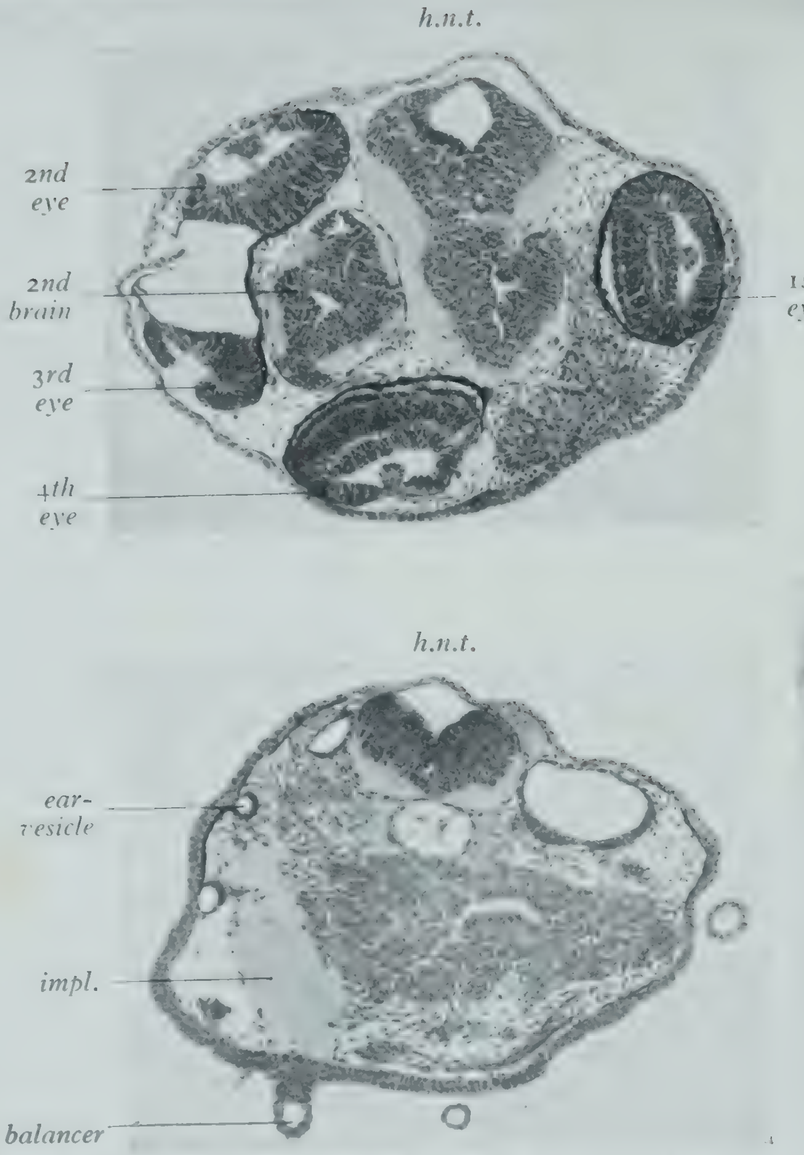

h.n.t.
2nd eye
2nd brain
3rd eye
4th eye
1st eye
h.n.t.
ear-vesicle
impl.
balancer

Fig. 88. Induction of chaotically arranged secondary embryo by implantation of boiled adult amphibian brain into the blastocoele cavity.

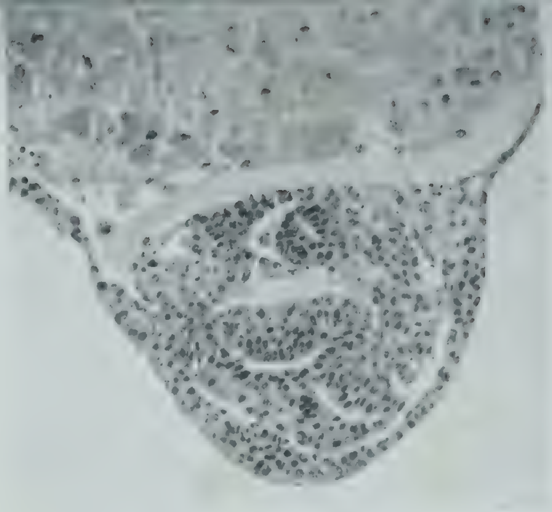


Fig. 89. Induction of secondary brain by implantation of chick embryo extract into the blastocoele cavity.

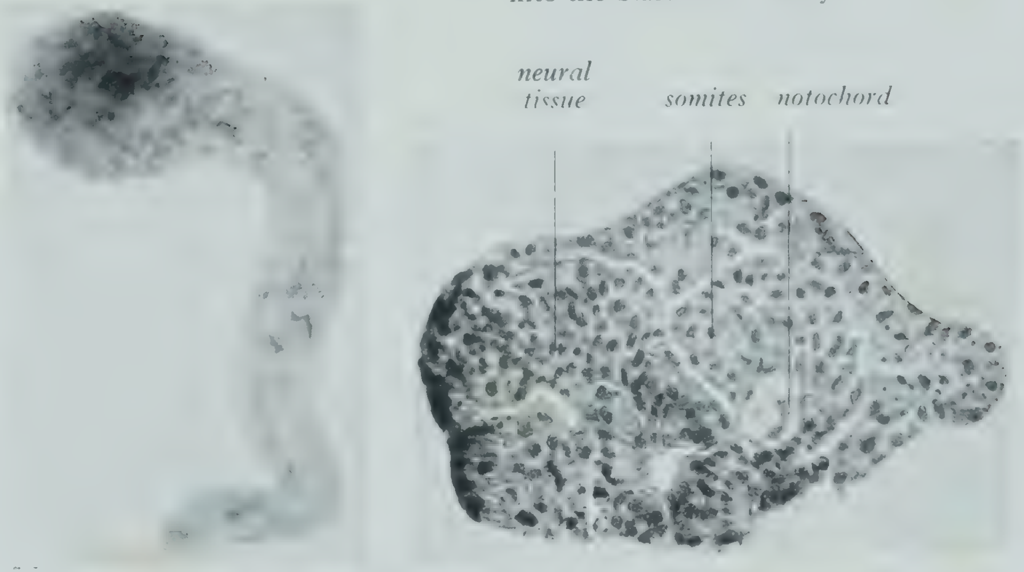

neural tissue
somites
notochord

Fig. 90. Induction of neural tube, notochord and somites by chick embryo extract in a competent ectodermal ball.

accessory brain with four eye-cups, two ear-vesicles and a supernumerary ancer. The curious phenomenon of lens induction without other structures appears; we shall return to this later on in connection with normal lens uction (p. 299).

ne inducing material proved itself particularly fertile in inducing effects (see g. 89), namely, 7-day chick embryo extract (in Tyrode solution). In explanted lates not only neural tissue but also notochord and somites were induced (see . 90). This was the nearest approach to induction by a living amphibian aniser. Among the minor points arising from this work was that no cific differences could be established between the different tissues or the erent phyla. Only in general it could be said that in those cases where the gest neural formations appeared there also was it most likely that mesodermal uctions, such as notochord or somite tissue, would occur. Mesodermal uctions were indeed frequent enough. Autolysis, as of adult newt liver, med to diminish the inductive power to some extent. Attempts to obtain uction by applying the stimulus from coagulated tissue to the outside of the petent ectoderm rather than to the inside, failed.

f much interest was the fact that Holtfreter[12] made a large number of implantans of plant materials, e.g. meal, potato, banana, agar, etc., always with negative lts. Wax was also without effect. Since that time there have been a number nvestigations of plant materials, notably by Ragozina, by Toivonen[1] and by da[2], but a study of their published photographs clearly shows that though they selves interpreted their ectodermal thickenings as positive results, the effects st still be regarded as quite negative.*

he existence of evocators in adult and late embryonic tissue furnished ddington[10] with a rather serious criticism against the work of an American ol (e.g. Hunt[1]; Willier & Rawles[1,2,3], etc.) which studied embryonic determina- in the chick by means of transplantations to the chorio-allantoic membrane. ointed out that just as the presence of active evocators in adult tissues ined the curious results which had been obtained on amphibian transplants re Holtfreter's introduction[2] of the neutral inorganic medium (see p. 157), so prived the chorio-allantoic situation of any right to be called neutral. He ced some inductions in amphibian embryos with chick blood in support of riticism. Although it was fully justified, there was yet a paradox remaining, n the chorio-allantoic membrane isolated pieces of blastoderm do not rentiate as fully as they do in the method of explantation on to a plasma clot. n this latter, too, induction might be expected, but does not actually happen. utral medium for bird embryos as well as for those of amphibia would be a t desideratum.

any of the foregoing facts were confirmed by Wehmeier (who had been onsible for the original experiment in which the inductive power was not royed by killing a dorsal lip with alcohol, published in Bautzmann *et al.*) in simultaneous with Holtfreter's. She obtained quite negative results with gelatin, cholesterol and potato starch; established that treatment of inductors

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ere is, of course, no reason why plant materials should not produce neural inductions if any m brought about the right kind of injury to the ventral ectoderm (see on, p. 181).

with organic solvents does not destroy their activity;* and obtained acti of non-inductors with organic solvents. She also investigated a number of organs, with results incorporated in Table 15, and confirmed Woerdem experiment[4] with tumour tissue. Certain tissues, such as amphibian retin rat carcinoma, were found by her to be a good deal more active after ac treatment than before, suggesting that in some cases the evocator is not per liberated in adult life. In her experiments there was a tendency for the form of enormous neural plates, with abnormal folds and delayed closure. The ch disposition of organs, etc. was just as in Holtfreter's work, save that few o embryos lived long enough to allow it to be well seen.

2·345. Chemistry of the primary organiser

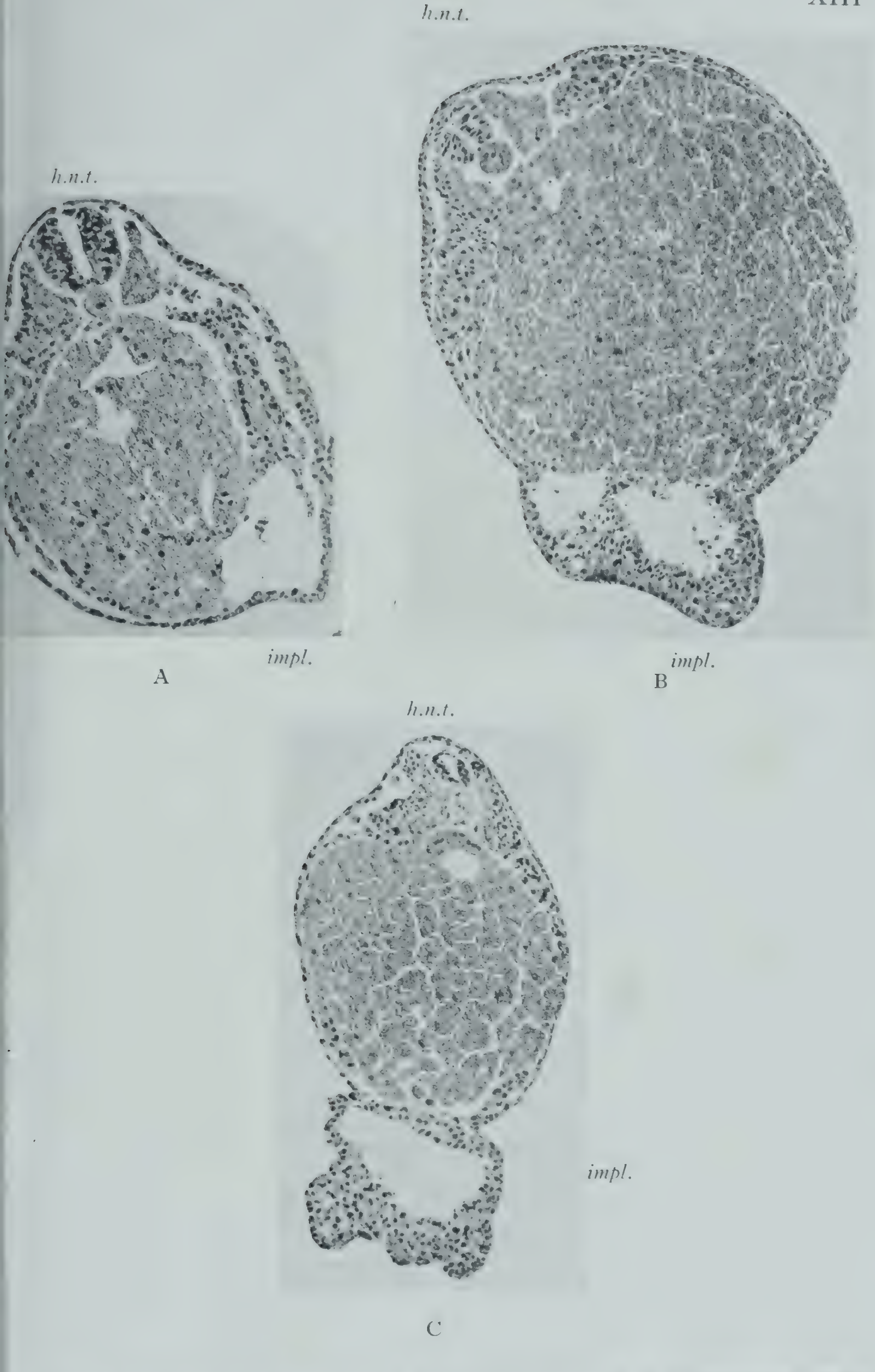
Cell-free extracts showing evocator activity were prepared almost si taneously by Needham, Waddington & Needham; Holtfreter[11]; and Spem Fischer & Wehmeier. The first group of workers ground newt neurulae in a s tube, using the interstitial water as the extraction medium, took up the mu looking liquid into a capillary, and centrifuged it. When the layers had separa the tube was cut with a file after heat coagulation at the junctions betw them, and the different fractions implanted. Neural inductions of moderate were obtained from the middle aqueous layer or the aqueous and the fatty l together. A similar technique was used by Holtfreter, who, using unfertil eggs, distinguished five layers; centripetally: (1) fatty layer, no inducti (2) brownish aqueous layer (probably a mixture of (1) and (3)), positive inductio (3) greyish aqueous layer, positive inductions, (4) black granular layer, not tes (5) solid substratum of lecitho-vitellin yolk-platelets (*CE*, Section 1·13), inductions. These results could hardly give any evidence about the solubilit the evocator, since the aqueous layer would be highly colloidal, but they indica that it was not bound up either with yolk-platelets or with fat-globules.

Spemann, Fischer & Wehmeier followed another method; they treated un tilised eggs with acetone and then extracted them with 20 % ammonium acet centrifuged out debris and precipitated with alcohol. The evocator was in precipitate.

From this point of departure different groups of workers were led in differ directions. It will be convenient before proceeding further to make quite c what is, or should be, regarded as a positive result in interpreting the eff produced by the implantation of chemical compounds. Lack of a uniform a reasonable standard has led some workers astray.

In the first place, the competent ectoderm may show no reaction at all. Fig. 9 shows a case, taken from Needham, Waddington & Needham, where, a implantation of a mixture of tripalmitin and triolein, no reaction of any kind found on the part of the ectoderm, which simply enclosed the implant in a pou On the other hand, there may be ectodermal thickenings of considerable s (Fig. 91 B), made up simply of cuboidal cells very like those of normal ectode These are most frequently lenticular, but they may form large irregular thickeni

* This was later confirmed again by Barth & Graff, who make it the basis of their convic that the primary evocator, and perhaps other inductors also, are of protein nature.



1. Effects produced by implantation of chemical substances into the blastocoele cavity. .. No reaction whatever. B. Ectodermal thickening C. Ectodermal thickening with errated edge (' sterol bump"). *h.n.t.* or *p.*, host neural tube or plate; *i.n.t.* or *p.*, induced eural tube or plate; *impl.*, implant.

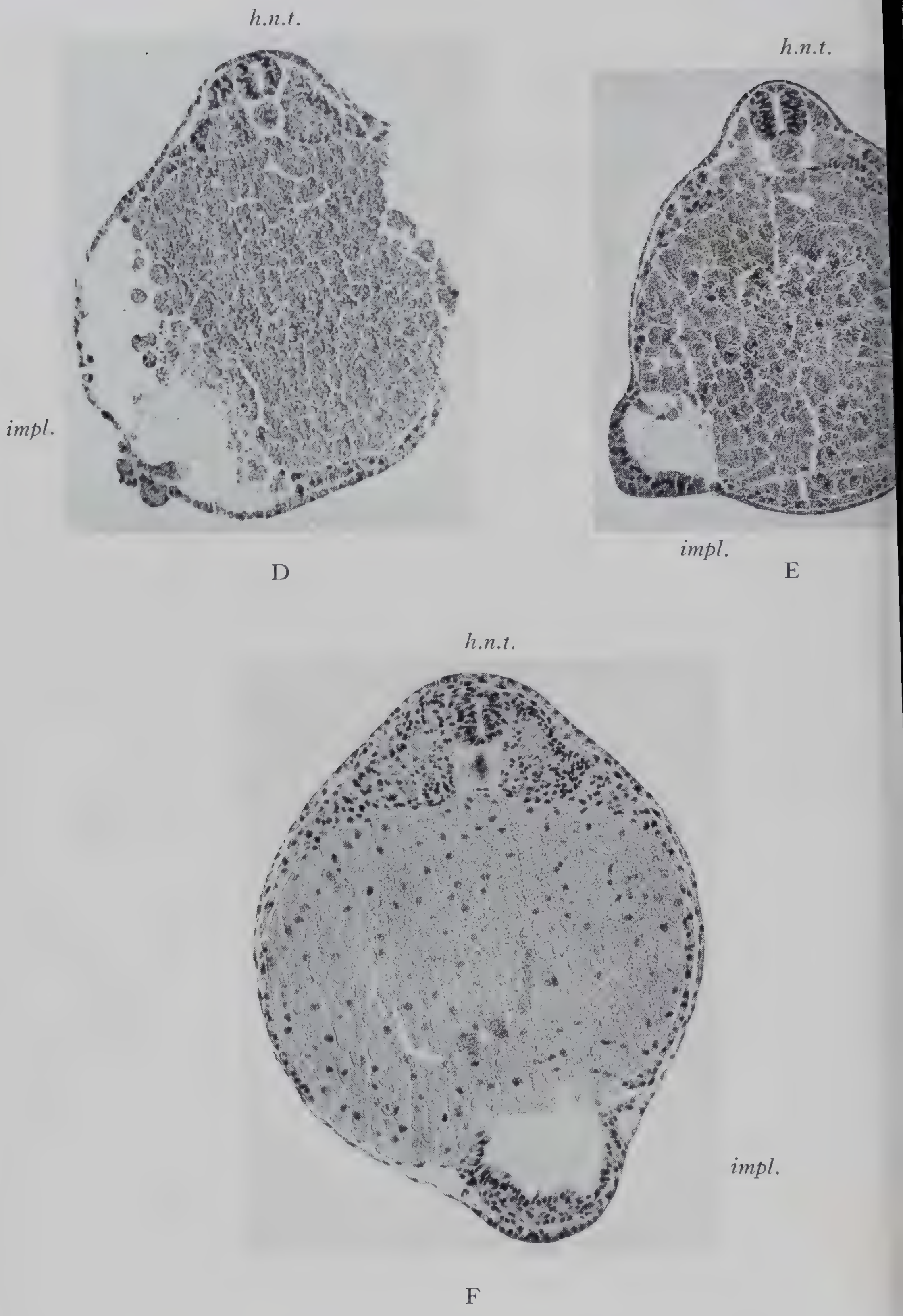

h.n.t.
h.n.t.
impl.
impl.
D
E
h.n.t.
impl.
F

Fig. 91 (*continued*). Effects produced by implantation of chemical substances into the bl cavity. D. Ectodermal reaction occurring just at the point of contact with the E. Weak "palisade" induction. F. Strongly marked "palisade" induction.

esenting a saw-like or serrated edge on the outside; they were associated by eedham *et al.* with the presence of non-effective sterols (such as phytosterol or ciferol) in the implant and were hence called "sterol bumps". An example is en in Fig. 91 C. In later development they form warty structures like those oduced by ultra-violet irradiation of the ventral epidermis of the tadpole lücksmann[2]). All these types show proliferation without differentiation. g. 91 D shows this taking place at a very localised region of the ectoderm iched by an implant of neutral fat and calciferol.

The first stages of neuralisation are seen in the so-called "palisade" inductions. re the implant is partly or entirely surrounded by histologically differentiated ural cells, columnar in shape, with elongated nuclei, tightly packed together form a one-layered or two-layered "palisade"-like epithelium. The nuclei are parallel to each other and to the main axis of the cells, usually in a row at the ne level. Such an induction may pass into a true neural tube as successive ial sections are examined, but this is rather rare. On the other hand, there may enough palisade neural tissue to make two or three neural tubes the same size hat of the host. Examples are shown in Fig. 91 E & F.

true neural tube is immediately recognisable, as also a neural plate in the of closing, by their characteristic cell arrangement. Examples have already n seen in Figs. 60 and 61. It is needless to say that care must be taken to inguish "*Spaltung*" effects, i.e. the mechanical diversion of the formative vements of gastrulation into two streams, by the implant or otherwise, from inductions. Higher phases of induction, such as the presence of recognisable in pieces, eyes with lenses, notochord, etc., usually admit of no mistake.

n this connection it is interesting that the mitotic rate in the induced neural e tissue is the same as that in the normal or host neural tube (Waddington & ikov; being 4·35 and 4·24 respectively as against 1·46 for ectoderm and 2·62 ectodermal thickening.

ome of the first investigators of the chemical properties of the primary evocator inclined to draw conclusions from the fact that dorsal lip pieces retained of their activity after being allowed to stand for hours or days in organic nts (e.g. Spemann, Fischer & Wehmeier), but the well-known difficulty of cting all lipoidal substances from protein material rendered no conclusion ble from such data (cf. the work of Macheboeuf & Sandor on extraction of s from blood serum, and the monograph of Macheboeuf). Needham, dington & Needham ground newt neurulae to a fine dry powder with drous sodium sulphate and extracted the mass with ether and then with l-ether in a micro-Soxhlet apparatus. Extraction was carried on for one day r.), a period quite insufficient to remove all the evocator, but which it was d would remove enough to give positive results, if the evocator was soluble ther. The ethereal extract was mixed with tripalmitin and triolein and anted. Positive results were obtained. Ethereal extracts of adult newt viscera gave positive results.

e subsequent experiments of the Cambridge group of workers were nearly ne using crystalline hen's egg-albumen as the implantation medium, ether- le material being dispersed in it by the acetone method of Boyland[1]. They fore carried out at various times a large number of control implantations which it appears that very weak neuralisations can occur from crystalline egg-albumen alone. Whereas there is generally no reaction at all (cf. Fig. there may occur neuralisations of a weak palisade type (cf. Fig. 91 E).* Co experiments are therefore necessary during each laying season, as the react of competent ectoderm varies from year to year. Without base-lines of this interpretation is impossible.

In the next paper of the series, Waddington, Needham, Nowiński & Lem obtained small neural inductions from the ethereal extracts of whole newt b and mammalian liver, and traced the activity into the digitonin precipita the unsaponifiable fraction. This final separation gave rise to a type of indu not seen in any other instance. While the filtrate from the digitonin precipita caused nothing but ectodermal thickenings of a quite negative character, precipitate induced rather beautiful palisade formations, which, like full ne tubes, were separated off very sharply from the ventral ectoderm, surrou the implant closely, and ended abruptly at the junction with the yolk-endo (see Fig. 91 F). Judged by the standard of reactivity that year these effects of unsaponifiable fraction were strikingly positive. Activity in the unsaponifi material from the ethereal extracts of mammalian liver was observed agai Waddington, Needham, Nowiński, Lemberg & Cohen in a later paper.

Though all these results, owing to the lack of facilities for sufficiently la scale routine testing, could only be of a preliminary character, the suggestion nevertheless inescapable that some substance of a steroid nature was indica This received considerable support from the finding of Waddington & Needh that fine neural tube inductions could be obtained by the implantation of cert synthetic polycyclic hydrocarbons. The first to give this result were 1 dimethylphenanthrene and 9 : 10-dihydroxy-9 : 10-di-*n*-butyl-9 : 10-dihyd 1 : 2 : 5 : 6-dibenzanthracene. A typical induction with the latter is shown Fig. 92 A. These two most active compounds had previously been known o as powerful oestrogens.

The series of polycyclic hydrocarbons and steroids which would act as prim evocators was subsequently extended by Waddington[21], who obtained neu tube inductions with the carcinogens methyl-cholanthrene, styryl blue (Fig. 92 and 3 : 4-benzpyrene (Fig. 92 C); and the oestrogens oestrone (Fig. 92 4 : 4′-dihydroxy-diphenyl (Fig. 92 E), 1 : 2-dihydroxy-1 : 2-di-α-naphthyl-a naphthene, and 5 : 6-*cyclo*-penteno-1 : 2-benzanthracene.† Particularly g effects were obtained with the water-soluble carcinogenic hydrocarbon sodiu 1 : 2 : 5 : 6-dibenzanthracene-endo-α-β-succinate (Fig. 93 A). The hydrocarb naphthalene (Fig. 93 C) and anthracene (Fig. 93 B), but not chrysene, gave sli positive results, possibly owing to the admixture of some impurity; as also perha for the same reason did the sample of cholesterol tested, though cholesterol h given negative results in the earlier work of Needham *et al.* Dihydrocholeste and equiline were absolutely negative, but inductions of lesser intensity occur

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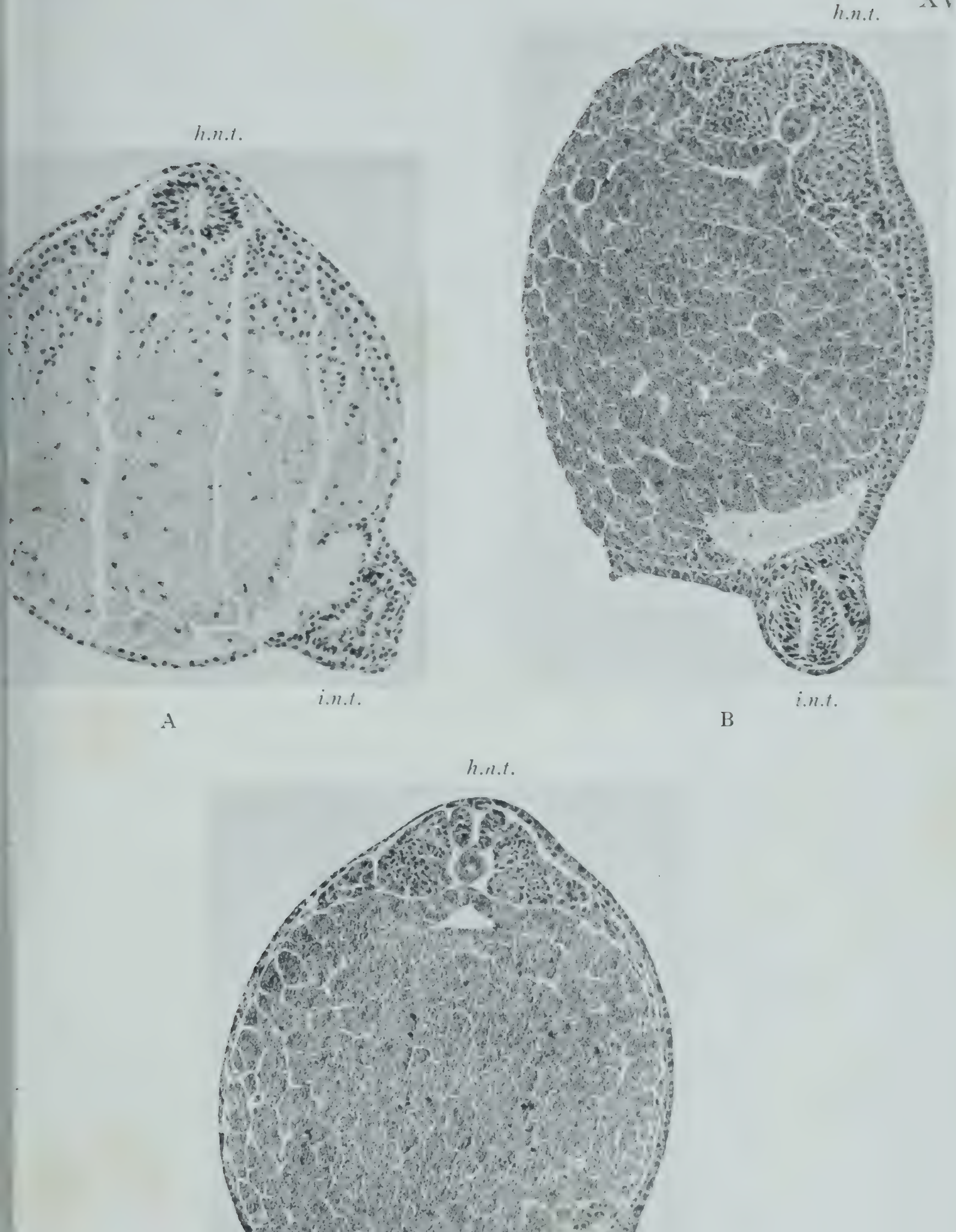
* Small palisades were also obtained from hen's egg-white, though not from egg-yolk, Wehmeier. If the purified albumen has stood for a long time, tubes may be induced, and older the specimen the more activity it has; this can be reduced or abolished by extracting with ether (Shen[2]). There is no change in the titratable –SH of the samples as they age (S & Needham).

† The formulae of all these compounds will be given later (pp. 245 ff.).



Inductions of secondary neural tubes produced by implantations of synthetic chemical substances the blastocoele cavity. A. 9 : 10-dihydroxy-9 : 10-dihydro-9 : 10-dibutyl-1 : 2 : 5 : 6-dibenzan- cene. B. Styryl blue. C. 3 : 4-benzpyrene. Conventions as in Fig. 91.

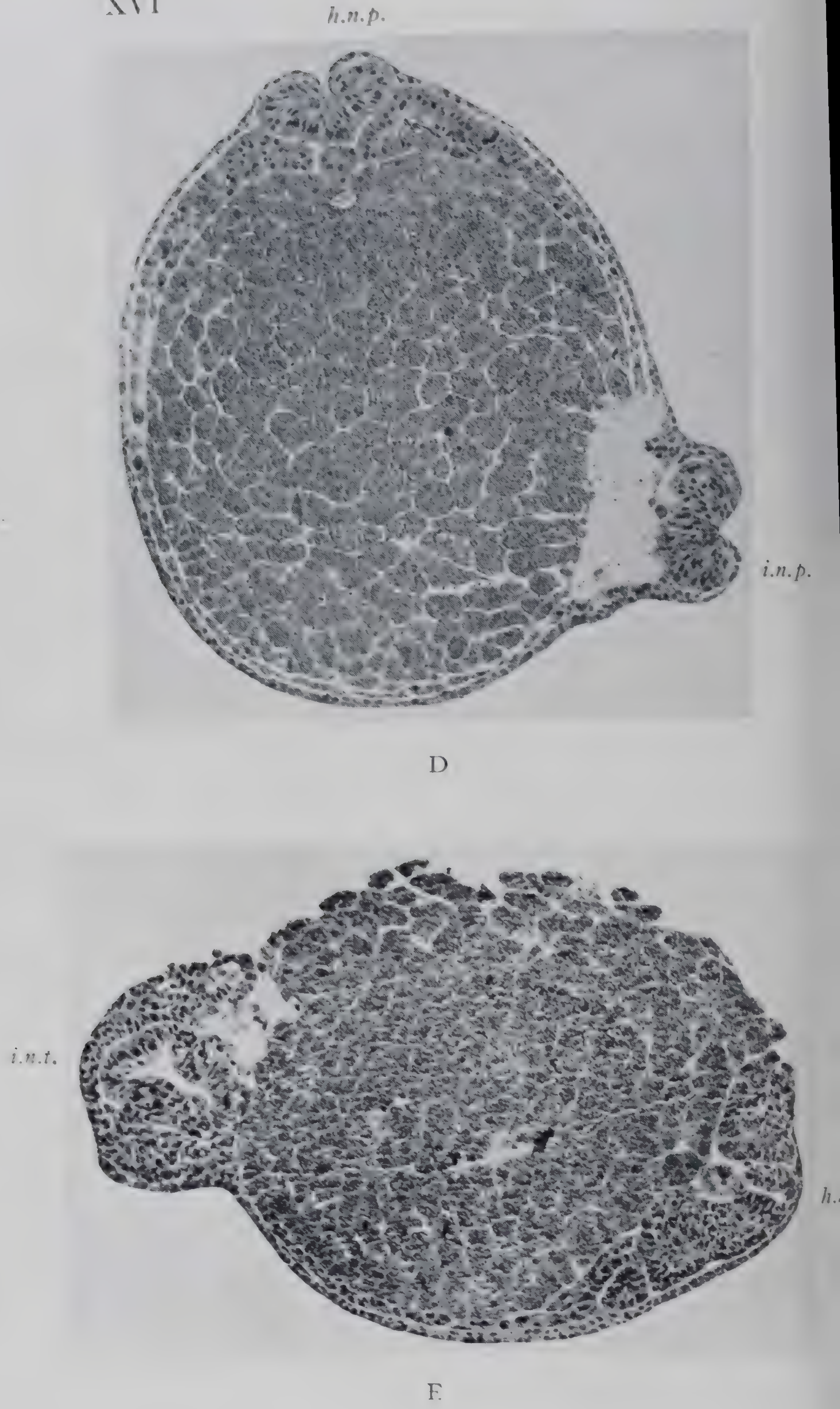
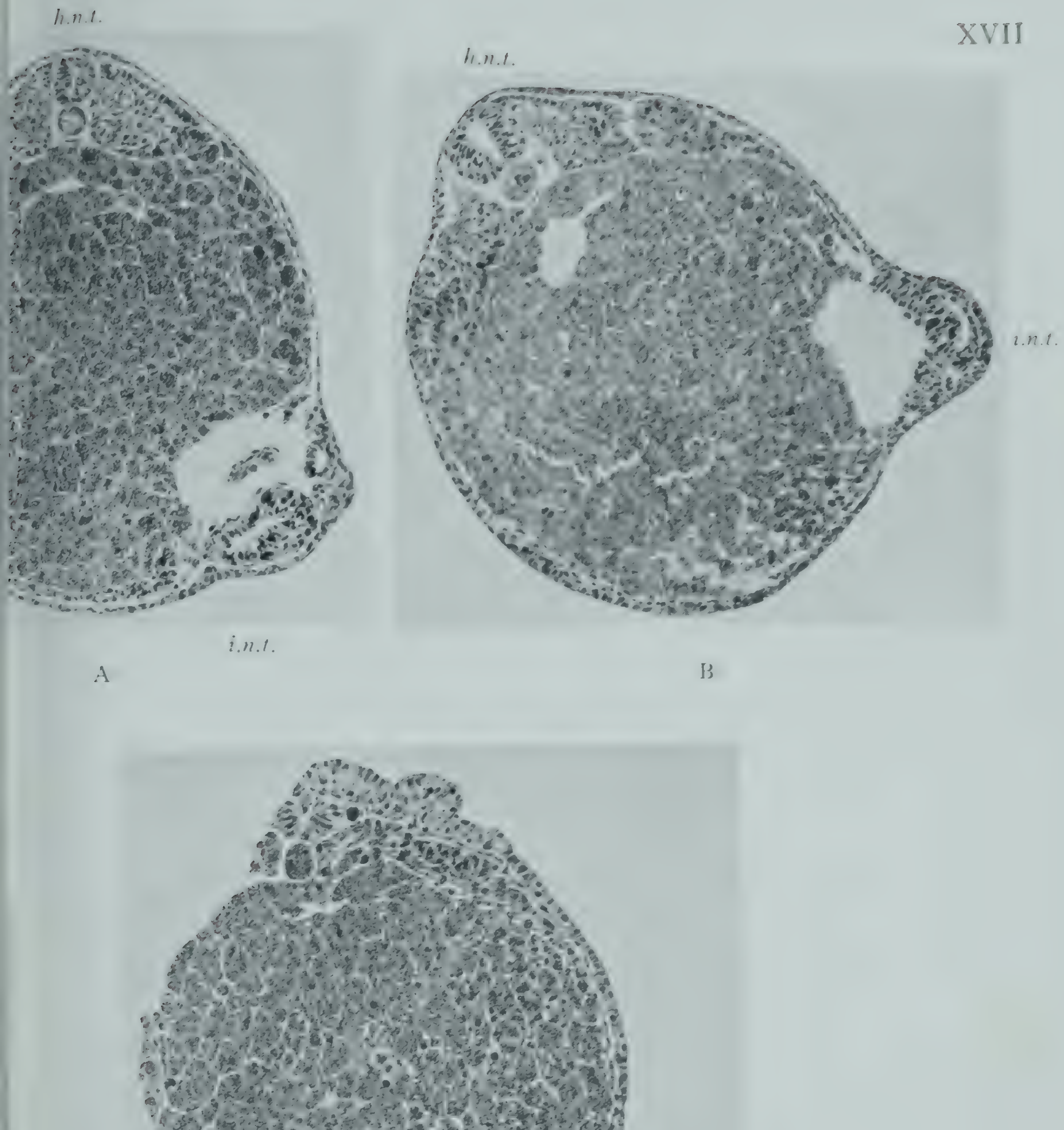

h.n.p.
i.n.p.
D
i.n.t.
h.
E

Fig. 92 (*continued*). D. Oestrone. E. 1 : 2-dihydroxy-1 : 2-di-α-naphthyl-acenaphthene.

XVII



luctions of secondary neural tubes produced by implantations of synthetic chemical substances
lastocoele cavity. A. 1 : 2 : 5 : 6-dibenzanthracene-endo-α-β-succinate. B. Anthracene (unpurified).
thalene (unpurified). Conventions as in Fig. 91.

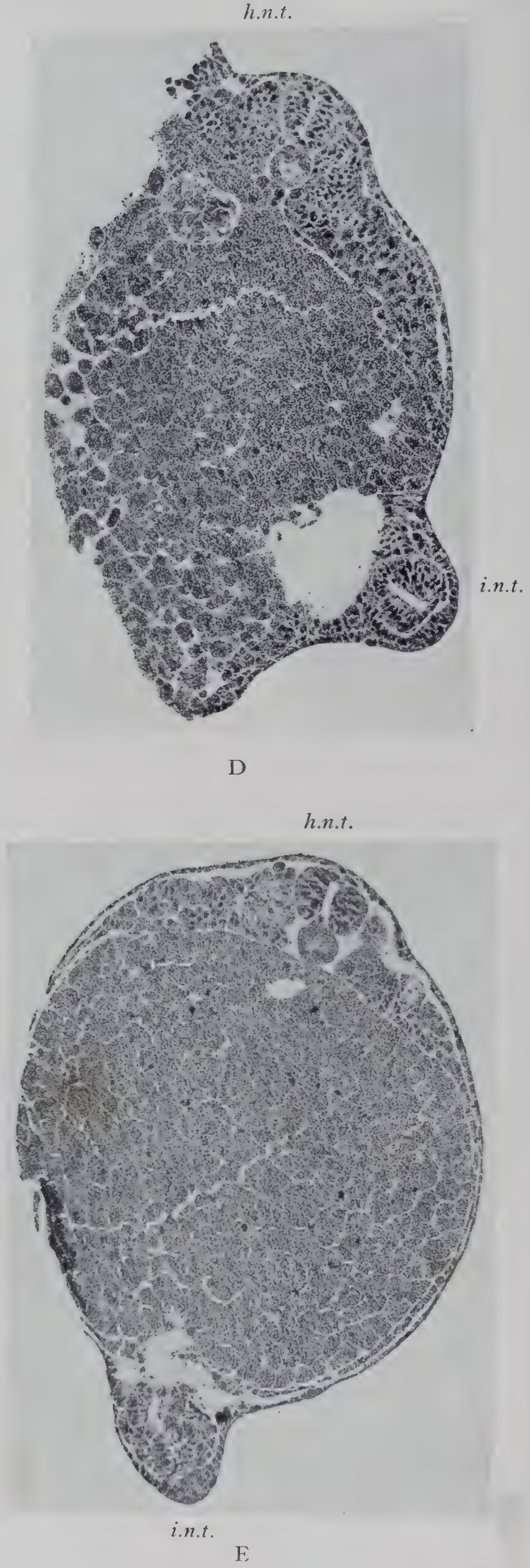

h.n.t.
i.n.t.
D
h.n.t.
i.n.t.
E

Fig. 93 (*continued*). D. Pregnantriol. E. Squalene.

n sitosterol, pregnandiol and pregnantriol (Fig. 93D) were implanted. The -isoprene compound, squalene, also gave inductions of low intensity. Though a polycyclic derivative, it is one from which such compounds might readily formed (Fig. 93E).

close relation was found in this work between frequency of induction in a es and magnitude of induction pro- ed in each embryo. Fig. 94 shows t the larger the percentage of neural uctions in the case of any substance, larger the amount of the neural tissue ned in μl. $\times 10^{-4}$. The only exception to was the carcinogen 3 : 4-benzpyrene, ch gave rise to a large number of ral tubes, all of which were very small. s not at present possible to elucidate meaning of these data, but on the le they may be taken as indicating action of a graded dosage of stimu- ry effect. We shall shortly see evidence (p. 183) that palisade inductions are eed the weaker forms of full neural tube induction.

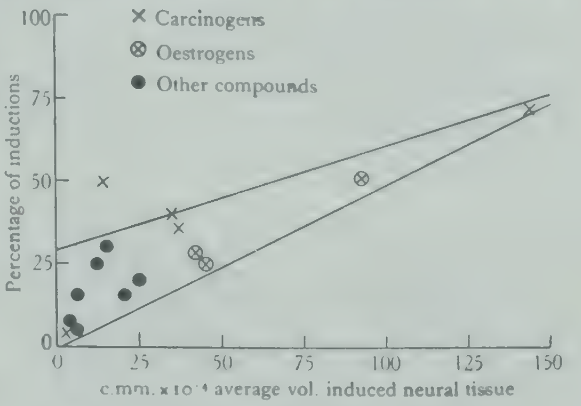


Fig. 94. Relation between frequency and size of neural inductions.

t is likely that we ought to attach considerable importance to the fact that ny synthetic polycyclic hydrocarbons and compounds of steroid character are ve in neural induction. The general biological significance of this fact will be cussed later (p. 243).

n the meantime other lines of research had been proceeding. Two workers the Freiburg group, Fischer & Wehmeier, had announced that glycogen, pared by the usual Pflüger method (i.e. by disintegration of the tissue with ng potash, followed by alcohol precipitation) would perform neural inductions. unfortunate that they never published any photographs of their sections, hat the extent of these inductions is not known, but later on Heatley, ldington & Needham successfully obtained neural tubes by implantation of ogen (see p. 278) and the fact itself is not in doubt. Not long afterwards, ever, it was shown by Waddington, Needham, Nowiński & Lemberg that if ogen, prepared in any of the usual ways, is extracted with ether in a Soxhlet aratus for 50 hr., a very active extract is obtained. The amount of solid erial in these extracts must have been very small, since on evaporation of the r the residue was hardly visible; nevertheless good neural tube inductions e produced, see Fig. 95 (opp. p. 190). However, the fact that the activity of cogen is due to an ether-soluble substance attached to it in small quantity s not affect the interest of Fischer & Wehmeier's result, which, as we shall provided an important clue regarding the nature of the inactive masking plex from which the evocator is normally set free in the dorsal blastopore lip. he paper of Fischer, Wehmeier & Jühling the suggestion that glycogen itself the evocating substance was withdrawn, since highly purified specimens e inactive. It may be added that the existence of a very active substance ched to glycogen furnishes additional indirect evidence that the evocator is an unsaponifiable compound, for had it not been so it would have been remo by the strong potash.

About the same time Barth[1] in New York found that beautiful induction brain tissue* could be had by the implantation of fairly crude kephalin prepa from mammalian brain. This phenomenon again appeared to be of the natur contamination of the product with some more active substance, since Waddingt Needham, Nowiński, Lemberg & Cohen noted that the activity of keph preparations (though in their hands small) was conserved after saponification hence destruction of the kephalin. Barth[2] subsequently reported further inducti with purified kephalin, however.

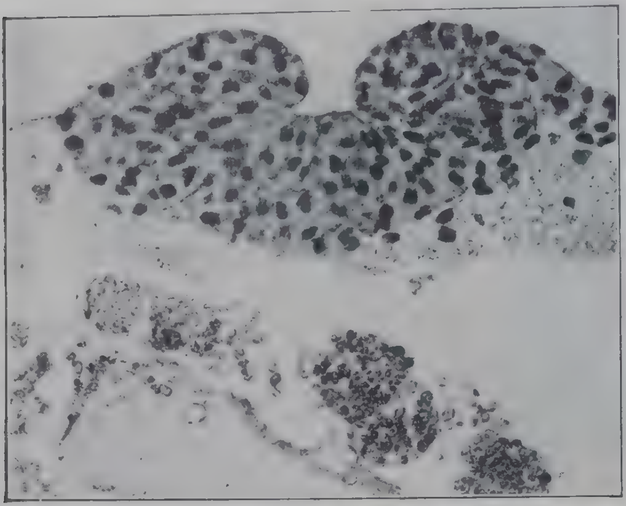


Fig. 96. Neural plate induced by implantation of synthetic oleic acid.

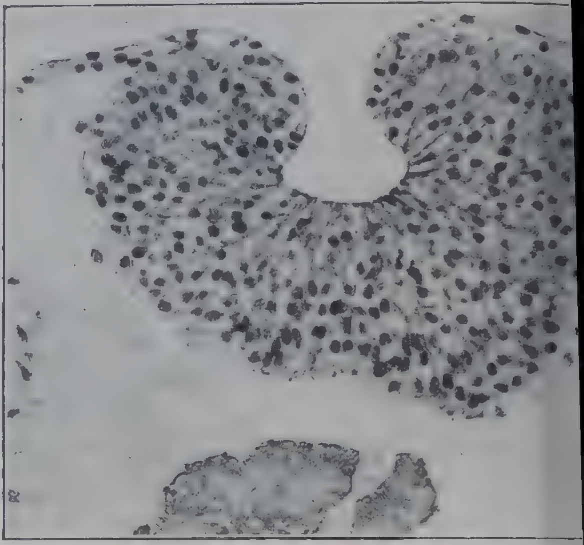


Fig. 97. Neural plate induced by implantati nucleoprotein preparation (thymo-nuclei

When Fischer, Wehmeier & Jühling announced that good neural tu inductions could be obtained by implantation of thymonucleic acid and of musc adenylic acid,† the subject began to enter a more complicated phase. This w followed up by the publication of Fischer, Wehmeier, H. Lehmann, Jühling Hültzsch, in which neural inductions were produced by many oils, but pa ticularly with oleic acid prepared from crystalline dihydroxystearic acid, an with linolenic acid, repeatedly purified through the bromide. Octadecenic aci prepared from ricinoleic acid, was also active, and so also oleic acid synthesis from sebacic acid and octyl alcohol. Here again no photographic evidence w published, but Fischer, in a separate communication, adduced two good neur plates, one the result of synthetic oleic acid implantation, the other the result the implantation of nucleo-protein from calf thymus. These are reproduced Figs. 96 and 97. Fischer *et al.* and Woerdeman[6] confirmed the finding of Needha

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* Much better than those actually figured by him, as I know from slides which he kindly allow me to examine.

† If this is true, it might be explained by an intervention of the ribo-purine, related as it is cozymase, in the course of carbohydrate breakdown, affecting the liberation of the masked evocat Subsequently, in the hands of Suomalainen & Toivonen, yeast adenylic acid (which, though differs from muscle adenylic acid only in the position of the phosphoric acid group, does not parti pate in the enzymic reactions of carbohydrate catabolism in animals) gave no neural inductio whatever.

. that active ethereal extracts of embryos or adult tissues could be obtained, the first-named investigators claimed that this activity was lost on neutralisa- of free acids in the extracts. This effect could never be obtained by the kers in Cambridge, in spite of several attempts. Water-soluble fatty acids and l fatty acids were not found to be active.

the opinion of Fischer and his collaborators, induction is brought about by stimulus of a large number of acid substances, owing their evocator power to r acidity. In this concept there is some obscurity, since the buffering power e tissues would be expected to neutralise acid ions. But it is clear that we to deal with complex phenomena, and especially with one rather deep-seated hodological difficulty, which has already been referred to (pp. 169, 174).

he difficulty may be stated in this way. We have already seen that the umptive epidermis which contains the masked evocator, and the competent derm which reacts to the stimulus of an artificially supplied evocator, are and the same tissue. Thus competence to form histologically neural tissue a morphologically recognisable neural tube cannot be separated from the tence of a masked evocator. Free evocator can exist without competence, as dult tissues, but the opposite condition has not yet been discovered. It is efore logically impossible to maintain, in any case where a given substance sed as the successful stimulus for an induction, that this substance stands in e chemical relation to the substance normally at work in the dorsal lip of the topore, for it can always be held that the substance tested simply unmasked ctivated the natural evocator already present in the competent tissue.* Thus, e diagram of Fig. 98, X is the implanted substance; it may act to give the onse of induction, R, directly, but it may also act by liberating the masked niser, (E), which then performs the induction. Analogous difficulties seem e not unknown in other branches of biology, for pharmacologists, according g, seek to account for the parasympathetic action of pilocarpin by supposing it stimulates cells, which usually secrete acetylcholine, to do so. Or again, ympathomimetic effect of tyramine and ephedrine is said to be due to the lation of the suprarenal gland by these drugs to produce adrenalin. The cation of oleic acid, therefore, to the competent ectoderm, might simply be re gentle way of setting free the masked evocator than boiling the tissue or g it in some other manner.

ere is one way out of the dilemma, however, namely a close attention to the tion of the minimal effective dose. We can probably accept the principle other things being equal, the larger the necessary dose of X, the more age will be done to the tissue, and the more likely it will be that the natural ator (E) will be unmasked; whereas the smaller the necessary dose, the nearer substance probably stands in chemical constitution to the evocator itself. e effective substances are considered in this way, it is found that while the ge of glycogen, kephalin, oleic acid or thymonucleic acid required to produce fect has been rather large, that of the ether-soluble sterol-containing fractions

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e early investigators of the hormones of adult mammals did not have to contend with a lty of this kind.

has been exceedingly small. Thus the active ethereal extract of glycogen alr mentioned was implanted at the strength of under 0·01 mgm. per c.c. of impl tion material (gelatin or egg-albumen or fat), while glycogen has been us about 200 mgm. per c.c. and thymonucleic acid much stronger.

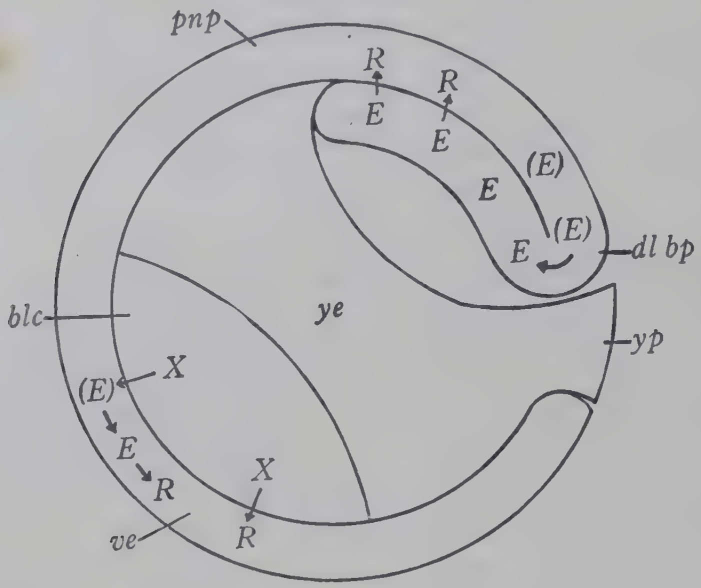


Fig. 98. Diagram to illustrate direct and indirect induction.

X,	chemical substance	*dl bp*,	dorsal lip of the blastopore
(*E*),	masked organiser-substance (evocator)	*pnp*,	presumptive neural plate
E,	free, active, organiser-substance (evocator)	*ve*,	ventral ectoderm
R,	response of neuralisation	*ye*,	yolk endoderm
blc,	blastocoele cavity	*yp*,	yolk-plug

The ectoderm is thickened for diagrammatic purposes.

This question was put to a direct test by S. C. Shen[1], who implanted the wa soluble hydrocarbon derivative 1 : 2 : 5 : 6-dibenzanthracene-endo-α-β-succin previously shown to be carcinogenic by Barry *et al.* and L. D. Parsons, into blastocoele cavity in carefully graded doses from 1·0γ to 0·0000125γ per emb The results are shown in Fig. 99.

The first noteworthy point was that the inductive action of this hydrocar showed an *optimal* dose at 0·0125γ per embryo. At this dilution the numbe neural tubes induced was a little over 40 % of the number of embryos in wh the implant was retained, but on each side of it the percentage of neural tu fell off. While it is not easy to picture the mechanism of the optimal dose ef in precise chemical terms, the fact that there is an optimal concentration ma it rather unlikely that the substance injures the tissue on which it is acting.* it did so, and liberated the masked organiser substance, its effect would expected to increase with concentration.†

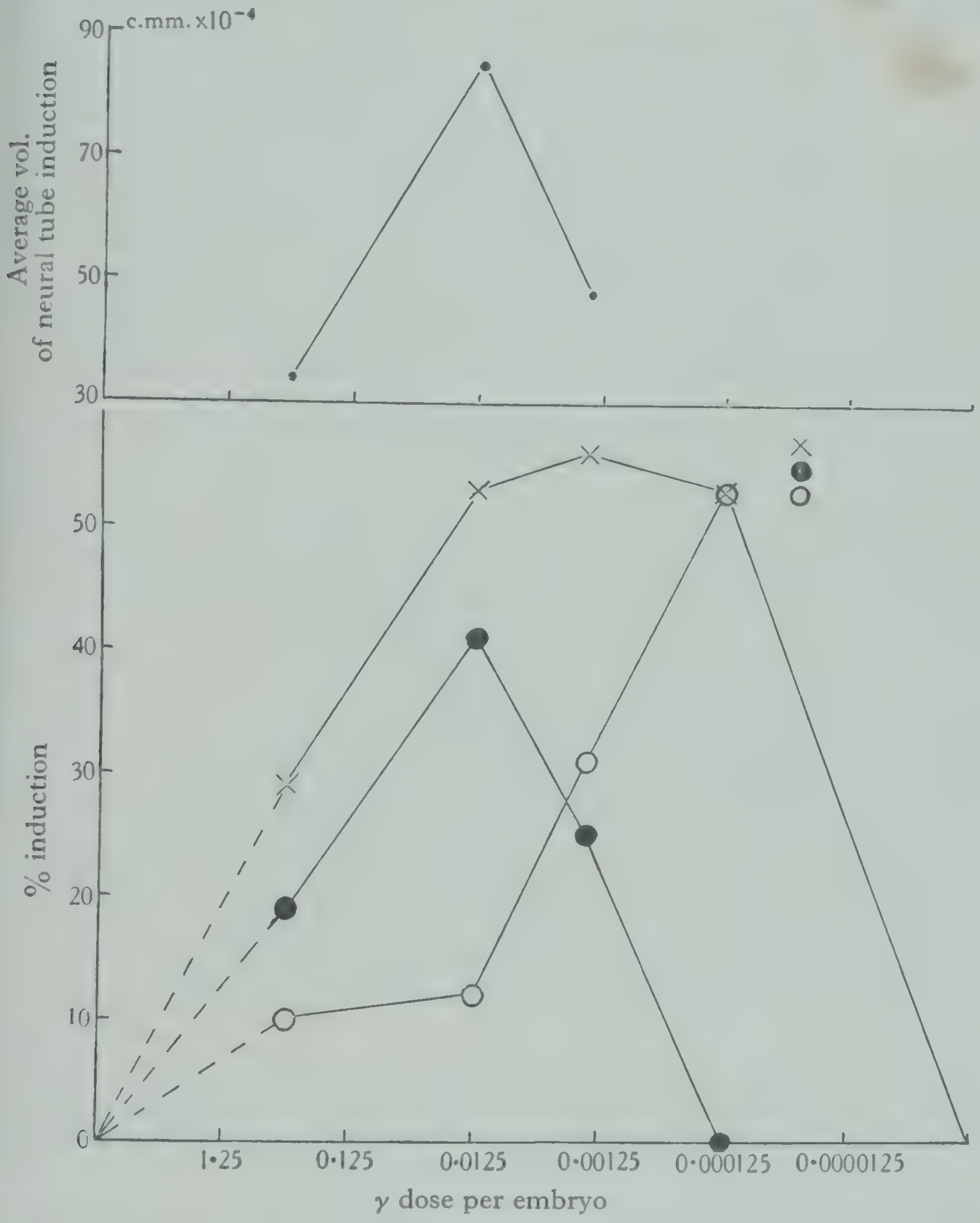
Another interesting point is that the palisade inductions rise to a maximu concentrations lower than the optimum for neural-tube inductions. This supp

* An examination of the slides showed no larger number of degenerating cells and pyc nuclei at the higher dosages than at the low.

† There is a parallel here in the work of Koltzov[5] on the production of polyploidy in plan colchicine (see p. 509), where an optimal dosage is also found.

impression hitherto generally current, that they are weaker manifestations e same process (see p. 177).

he parallelism between frequency and size of inductions noted by Waddington[21] found to hold good in Shen's work also; it will be seen from Fig. 99 that the age volume rises on each side of the optimal concentration to a peak at l. × 10^{-4}. This level compares with the largest volumes of Waddington's series.



. 99. Relation between dosage, induction frequency and size (with a water-soluble hydro-
arbon derivative). × all inductions, ● neural tubes, ○ palisades.

rhaps the most important point about Shen's investigation, however, is the level of dose required to produce neural inductions. It is instructive to late the doses required in mgm./kg. wet wt., and to compare the resulting es with others in the literature for various biological responses. Assuming a newt gastrula weighs approximately 4 mgm., we find from Fig. 99 that naximum neuralising activity (including both neural tubes and palisade inductions) occurs when the gastrula receives 0·001 γ. The volume of the blas coele cavity is taken as $\frac{1}{4}$ of the egg volume, namely 1 c.mm., and the implantat mass $\frac{1}{4}$ of the blastocoele cavity. Such a dose corresponds to 0·25 mgm. wet wt. Effects are still obtained, however, at a dilution one hundred times lo i.e. 0·0025 mgm./kg. wet wt.

This range stands in some contrast with the dosages which have been emplo by some of the workers who have implanted chemical substances. Thus Fisc *et al.* used 5 % free fatty acids emulsified in agar-agar, so that 1 c.mm. wo have contained 50γ fatty acid, i.e. a dose of 3·15 gm./kg. wet wt. Their nucl protein preparations, moreover, had to be implanted as such, so that each gastr must have received about 250γ, or 62·5 gm./kg. wet wt. Later Barth[2] repor effects (neural-tube inductions?) using 0·05 % digitonin in egg-albumen, i.e dose of 31·5 mgm./kg. wet wt. It will be seen that these amounts are all of order of a hundred or a thousand times the dosages used in Shen's work. T latter, moreover, fall within the range of activity of hormones, vitamins, and ot stimulating substances, for which a few data are assembled in Table 16. T

Table 16. *Activities of biological stimulating substances*

Biological response reactions	Investigators	Effects and agents		Effecti dose mgm./k wet wt
Neural inductions	Shen[1]	1 : 2 : 5 : 6-Dibenzanthracene-endo-α-β-succinate:		
		Maximum effect		0·25
		Minimum effect		0·0025
	Fischer *et al.*	Fatty acids		3150
		Nucleoprotein		62500
	Barth[2]	Digitonin		31·5
Hormone action	Went & Thimann	Minimal effective doses:		
		Oestriol	Mouse	0·1
		Androsterone	Capon	0·1
		Insulin	Rabbit	0·25
		Thyroxin	Man	0·01
		Histamine	Cat	0·01
		Auxin	Oat plant	0·0001
Drug action	Chen, Chen & Anderson	Digitalis principles, minimal effective doses on heart:		
			Cat	0·2–8·3
			Frog	0·0007–
Oestrogenic action	Cook, Dodds & Lawson	9 : 10 : Dipropyl-9 : 10-dihydroxy-dibenzanthracene		0·25
	Dodds, Golberg, Lawson & Robinson	Diethyl-stilboestrol		0·004
		Oestrone		0·016
Vitamin actions	L. J. Harris[3]	*A* Daily requirement, man		0·016
		B_1 Daily requirement, man		0·011
		C Daily requirement, man		0·39
		D_2 Daily requirement, child		0·0018
Carcinogenic effect	Deelman	1 : 2 : 5 : 6-Dibenzanthracene minimal dose yielding tumours in 34 weeks:	Mouse	0·6
	Shear[1]	1 : 2 : 5 : 6-Dibenzanthracene minimal dose yielding tumours in 14 months:	Mouse	0·016

N.B. (1) The inductions with digitonin were always of the palisade type, and apparently much more pronounced than those of controls in which egg-albumen is implanted.

(2) The figures for Fischer's inductions were by a slip printed wrongly in the original ta

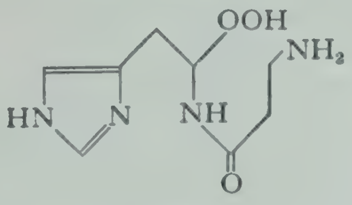
nparison between these is, of course, a little difficult, since hormones and drugs injected in single doses, the vitamin figures are for daily requirements, and carcinogens, which usually work very slowly, are administered by successive ections. However, the main point, that the dose of water-soluble hydrocarbon ducing the maximum inductive effect on the gastrula ectoderm lies in the ie range as that of many biological stimulating substances, is clearly established. ince this is the case, it would seem more likely that the action of this substance d hence, by implication, of the other hydrocarbons active in neural induction) *irect*, that is to say, in imitation of the normal organiser, rather than *indirect*, liberating the organiser substance masked in the ventral ectoderm.

)n the other hand, it is equally likely that some results, such as the rather ak inductions reported by Okada[2] on implantation of kaolin, silica, etc., and se reported by Barth[2] on the implantation of very acid and alkaline jellies I 3 and 10), are to be interpreted as indirect inductions. In the former case able numbers of degenerating cells were observed in the vicinity of the plant, and Okada himself explained his effects in this way.*

Jnfortunately, the force of arguments about dosage has been somewhat akened by the remarkable results of the plant physiologists who investigated hormone, auxin, which causes growth by the elongation of the cellulose cell-lls. Auxin, as isolated from the higher plants, is a cyclo-pentene derivative, five-membered ring of which has one double bond and three branched side-ins (see below, p. 266). Its most interesting relative is the constituent of the i-leprosy chaulmoogra oil, chaulmoogric acid, in which, however, the ring is minal to a long chain. Hetero-auxin, however, as isolated from yeast, is ndol acetic acid, a substance allied to the common amino-acid tryptophane. t, according to Kögl, hetero-auxin is active in some cases in only half the dose auxin itself, though it produces exactly the same reaction of cell growth.

Ve have not here the difficulty, however, which would arise if the test-object ained a sufficient amount of *easily liberated* masked auxin to give the reaction p. 212). Furthermore, hetero-auxin is not contained in the higher plants. nother case is that of the stilbene derivatives (see below, p. 256), which are ctive oestrogenically as the hormone of the ovary itself. In spite of all, ever, the establishment of the minimal effective dose remains a matter of it importance.

ductions have been claimed by Finkelstein & Schapiro[2] for carnosine (β-alanyl-histidine). ke most workers, they paid attention to dosage, and had 14 % positive results at 0·01 γ per ula. Only two illustrations are offered, however, in support of this; one shows a mere ecto-al thickening, and the other an oblique section cutting both the cephalic and caudal ends of



Carnosine

neural axis, one of which was perhaps mistaken for an induction. Though it does not seem y that carnosine is effective, there would be no harm in testing it again. Tests of vitamins and nones by Suomalainen & Toivonen were all negative. The effects of autolysis or proteases on ie containing the masked evocator have not yet been investigated.

Until recently the suggestion that the higher fatty acids liberated the evoca
in the ectoderm by a generalised injury less severe than the denaturation of
proteins was the only one possible. But it has been shown by both the Amsterd
and Basel schools that testosterone, the active substance of the internal secreti
of the testis, closely related to the androsterone which appears in the urine,
dependent for its action on another factor. Its full activity is only attained wh
it is "activated" by certain substances, among which higher fatty acids, such
elaidic, oleic, crotonic, and especially palmitic acids, are prominent. Opti
action occurs at C_{16}. The activation seems to be unspecific, and that the phe
menon is not one of increased absorption through the intestinal wall can
demonstrated, according to Miescher, Wettstein & Tschopp, by injecting t
testosterone on one side of the body and the higher fatty acid on the other. T
whole parallel is important, for it supplies a new hint of a relationship betwe
the fatty acids and steroids where stimulatory substances are concerned.
should, moreover, not be forgotten that, as will be seen later (p. 248), oleic a
may possibly itself be a precursor of the sterol ring system.

To summarise, then, it is clear that neural inductions can be brought abo
by a wide variety of chemical fractions and pure or relatively pure substanc
Which, if any, of these, is identical with the primary evocator occurring in t
dorsal blastopore lip, and upon which the whole normal process of developme
depends, is as yet an unanswered question. The question is rendered particula
difficult owing to the presence of the natural substance in masked conditi
in the very tissue on which alone the activity of a chemical substance can
tested. Studies of dosage, however, suggest that the natural evocator is a stero
substance, for apart from the direct evidence from solubility, etc., the on
substance which has so far been shown to act in concentrations of the vitamin
hormone order is a polycyclic hydrocarbon. Many of the other types of substan
which have given positive results have probably done so by unmasking the natur
evocator. The next step ought to be the isolation of the intact complex itself; th
alone will allow of the identification of the natural evocator. Here the ultr
centrifuge may be expected to give important help.

There has sometimes existed a certain tendency (e.g. in Spemann[17], pp. 2
369) to abandon too readily the search for logical consistency in the chemic
facts, and to write off the whole induction effect as "unspecific". This is a couns
of despair. Whatever the ultimate value of the facts so far accumulated, there
one thing of which we can be sure, namely that throughout normal developme
the evocators of the different grades are at work, and the understanding of the
mechanisms must be mainly a matter of time. It will not be reached witho
much more knowledge than we have at present about the characteristics
metabolism in the various regions of the gastrula during the critical time
evocation and individuation. This will be the subject of the succeeding sectio

The above interpretation of embryonic inductions by chemical substanc
differs somewhat from views advanced by other authors such as Woerdeman
Woerdeman's main thesis was that the dead implants or chemical substanc
activate the cell material in some way, but that the production of the supe
numerary embryonic axis is the work of the host itself, which is capable of reacti
in this way to any stimulus. There were really two ideas involved in this co

sion: (1) that the host plays an essential part in the "induction" by a dead aniser, and (2) that the stimulus which proceeds from the implant is merely indifferent activation.

Vith the first statement Waddington & J. Needham in their commentary agreed; eed the first mention of this in the literature had been made by Waddington[6] in nection with the action of boiled organiser in the chick embryo. But they nted out that they had distinguished two aspects of the host's influence; on one hand, the determination of the histological character of the induced tissues the particular kind of reactivity present in the ectoderm submitted to the ucing stimulus; thus there is only a certain period of development during which host ectoderm will react to an inductive stimulus by the formation of neural ue (*Competence*); and on the other hand, the exertion by a living organiser nfluence on the regional character of an embryonic axis induced in its neigh- rhood (*Individuation*). It is certain that we must take into account the possible ividuating influence of the host's organisation centre on embryonic axes uced by dead implants.

he advantage of analysing the host's influence into these two aspects is a gain lexibility. Thus we can envisage the possibility of phenomena which involve npetence but not individuation. Some of Holtfreter's inductions[16] by dead terial in ectoderm removed from the host are an example (see p. 277), but a ter one still was attained when Heatley, Waddington & Needham found similar uctions performed by a chemical implant (see p. 278). Woerdeman got into e difficulty in interpreting Holtfreter's results. The basis of his theory was t the dead organiser activates a "field" already existent in the ectoderm, but also suggested that the fields are somehow bound up with the host's organisa- n centre and are therefore absent from the ectoderm in question, or at best y weakly developed in it; hence he was left with no explanation for the regular earance of induced neural tissues in Holtfreter's explants. Here the two cepts of competence and individuation carry us further. The regular forma- n of induced tissues indicates that the competence of the ectoderm is normal, le the contorted and irregular shape of the inductions points to the weakness e individuating influences. Further work is required before we can say ther isolated competent tissue retains some power of individuating into organs tissues which may be induced in it, but the experiments of Holtfreter make ely that the power, if any, is small.

he reverse of the above case is found when a piece of tissue individuates but not exhibit competence; a normal induction by a living organiser provides xample, since the living organiser does not itself realise any competence for formation of neural tissue although it does individuate the neural tissue which duces. Here we are not concerned merely with the activation of a "field" in erdeman's sense, but are bound to consider also the determination of the onal character of the field; and it is exactly this determination which we call viduation. Finally, it may be mentioned that Holtfreter's experiments with splants of ectoderm on to older larvae (see below, p. 274) have shown that at age when the competence to form neural tissue has entirely disappeared from host ectoderm, a capacity to determine the regional character of induced ral tissue still persists; here again the single process of the activation of a field, ken of by Woerdeman, has been broken up into two phases.

he second part of Woerdeman's thesis, namely, that the dead organisers vide merely an indifferent activating stimulus, is still more open to criticism. we have seen, substances have been described (such as certain sterols) which ulate the ectoderm to intense proliferation but which have not yet given rise to induced tissues. The hypothesis that in more favourable cases they also w act as inductors remains a hypothesis, and one which does not seem plausi unless some more cogent reasons can be adduced in its favour.

Moreover, the alleged induction of neural tissue by thermal or mechani stimuli rests on a very slight foundation. There are only two papers in which is asserted. Gilchrist[1] and Castelnuovo exposed embryos to differential therm gradients. Gilchrist's pictures show only irregular puckerings of the ectode the neural nature of which is uncertain, and one "*Spaltung*". Illustrations Castelnuovo's results were never published, but she describes her positive ca as "unite caudalmente", which strongly suggests "*Spaltungen*" and not prop inductions. Attempts have recently been renewed to bring about induction cauterising ventral ectoderm, but the results of Cohen[2] are extremely dubio and Margen & Schechtman report consistent failure to achieve any neur differentiation by mechanical injury. The only convincing evidence of inducti by an indifferent stimulus (implantation of celloidin) concerns quite a differe process, the formation of limbs (Balinsky[2]), and this, though so often quoted, re only on one single experiment (see p. 305). While we must not dismiss absolute the possibility that competent ectoderm can form neural tissue in response several kinds of stimulus, the more likely hypothesis is that it reacts like oth physiological systems which are sensitive to one chemical substance or to a limit class of chemical substances.

The word *Evocation* carries out several useful functions. In the first plac if we separate from "organisation" in general the concept of "regional dete mination" or "individuation" we require a word for what is left. This was one time called "induction as such", but is now spoken of as "evocation" Secondly, we give the name "evocators" to all substances which are known t stimulate gastrula ectoderm to neural differentiation, while thirdly, we can spea of the substance which is active in normal development as "the evocator". Som grounds for preferring, instead of the word "activator", a specially invente word, have been given above. But there is an additional reason, namely, that "th evocator" exists in the egg before gastrulation in a masked condition, from whic it can be liberated by the action of heat or by less drastic treatments. The ter *Activation* is here used to describe this liberation.

In an interesting review Weiss[14] too has critically discussed the organise problem. Weiss emphasised the significant difference between live and dea organisers and clearly distinguished between the "evocating" activity of th inductor and the "individuating" activity of the host field, "all the organising i.e. pattern-determining, effects going to the credit of the latter". "It appears" he concludes, "that this is the only stand one can safely take in view of such fact as have come to our knowledge so far" ([14], p. 667). With this we may agree, sa that such a formulation of the facts, like that of Woerdeman[6], fails to distinguis clearly between competence and individuation.

2·35. Morphogenesis and metabolism

2·351. Metabolism of the regions of the gastrula

To gain a clear understanding of the mechanism of activation of the primar evocator in normal development (liberation from a protein complex) we nee to know as much as possible about the metabolism of the various regions o known embryological properties in the gastrula. Until a few years ago, this wa impossible, owing to the delicacy of the necessary technique.

convenient transition is provided by the fact that it has proved possible to
ate the evocator by methods less drastic than denaturing the tissue-proteins.
piece of ventral ectoderm is isolated from the gastrula and cultured for a day
o in Holtfreter solution containing a small amount of a dye such as methylene
, it will on implantation into the blastocoele of another embryo manifest
cing power (Waddington, Needham & Brachet). The graft may also become
alised itself, since it possessed competence before it acquired inducing
er. In one case, after 2 days in $M.10^{-5}$ methylene blue, the graft
ced a large brain in a host and at the same time was converted itself into
mpact mass of neural tissue. It might thus be expected that if the isolate
cultivated longer in the methylene blue solution it would become neuralised,
this was subsequently found to be the case by Waddington (described in
dham[30]) and by Beatty, de Jong & Zieliński (Figs. 100, 101, opp. p. 190). In
rdance with the classical work of Barron and his collaborators on the increase
spiratory rate caused by reversible oxidation-reduction indicators (Barron
amburger; Barron & Harrop; Barron & Hoffman), this was at first associated
a metabolic acceleration brought about by the dye. It was later shown,
ever, by Beatty, de Jong & Zieliński that methylene blue has a relatively
l effect upon the oxygen uptake of ventral ectoderm (45 %) and that in all
ability other dyes, such as neutral red or janus green, which have a much
r oxidation-reduction potential than methylene blue and have never been
n to affect cell respiration, may also activate the primary evocator. A more
able hypothesis, therefore, may be that these dyes compete with the evocator
ts protein carrier, displacing it and so liberating it in active form. Dye-protein
plexes have been studied by many workers (e.g. Marston; M. R. Lewis[1] and
is & Lewis on viruses; W. W. Smith & H. W. Smith; Commoner on un-
lised *Chaetopterus* eggs). The action of dyes on the physiological and embryo-
il properties of ventral ectoderm would repay a good deal of further study.
her substances were also investigated by Waddington *et al.* and Beatty *et al.*,
as dinitro-*o*-cresol, a stimulant of glycolysis (see p. 569), but the few
iments gave mainly negative results. No inductions had been obtained when
elstein & Schapiro[1] had implanted this substance into the blastocoele cavity,
Dawson[3], applying it to the intact amphibian embryo during the earlier stages
velopment, obtained no effects other than a number of persistent yolk-plugs.
ffects on teleostean embryos are slighter still (Waterman[6]).

itial steps towards the elucidation of special metabolic characteristics of the
al lip region were made by histochemical methods. The earlier papers on
subject, e.g. those of Konopacki & Konopacka or Hibbard[1], were entirely
logical and so did not help this particular problem. The first investigation
haracteristics of regions seems to have been that of Voss, who found that
e the ectoderm of the gastrula, as well as presumptive mesoderm and
umptive neural plate, gave a strong "plasmal" reaction, there was none
ever to be seen in the roof of the archenteron immediately after invagination.
e substance, plasmalogen, responsible for this reaction* now turns out to

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Discovered by Stepp, Feulgen & Voit; cf. the reviews of Feulgen and of Grevenstuk.

be a special type of phosphatide in which aldehydes of higher fatty acid combined with glycerol, phosphoric acid and amino-ethyl alcohol (Feulg Bersin; Feulgen, Bersin & Behrens; Bersin, Willfang & Nafziger), it may be the dorsal lip of the blastopore is the seat of considerable changes in metabolism. The connection of plasmalogen with cytoplasmic nucleotides p. 269 and p. 636) forbids us to dismiss it as unimportant although its fun still remains obscure.

A clearer line of work was begun when Woerdeman[2], using histoche methods, observed a similar disappearance of glycogen from the dorsal l *Amblystoma* embryos during invagination. The division between the glyc containing cells of the presumptive mesoderm and the just-invaginated glyco poor cells was so sharp as to warrant the use of the term "*Glykogen-gren* An example is shown in Fig. 102.* He noted the relation with the organisa centre, and in a second paper[3], continuing work with the same methods, f a diminution of glycogen in the optic cup at the time of induction of the He was thus led to propose some causal connection between glycogen breakd and inductions, whether of first or second grade. The subject was then take by Raven[1], also of Amsterdam, who transplanted a dorsal lip from one embry the ventral part of another. In the host it invaginated as usual, taking with certain amount of host ectoderm (the process studied by F. E. Lehmann[3]), on sectioning, showed a glycogen boundary indistinguishable from that in normal invagination. In some cases invagination had proceeded so far this boundary lay not in the implanted organiser but in the host ectod experimentally made to invaginate in this way. The conclusion that some ca connection, and not a mere chronological correspondence, existed betw glycogen-loss and invagination was further strengthened[3] by the implantatio presumptive epidermis into the dorsal lip, where, as we have seen, it acqu inducing power and later forms notochord and somites. In such pieces also th was a marked glycogen-loss. On the other hand, pieces of presumptive epider implanted into the blastocoele cavity never showed any diminution in histochemically detectable glycogen; the effect was not due, therefore, to rem of tissue from the surface to the interior of the gastrula. In a further pa Woerdeman[4] noted that in standard inductions produced by implantation of liv dorsal lips, the inductors had always lost a large part of their glycogen. The tr of thought that glycolysis was concerned with induction led him to make experiments with muscle and tumour tissue already referred to (p. 171), thou with glycogen he had no success.

Lastly, it is not without interest, in connection with the disappearance b of glycogen and plasmalogen from the dorsal lip, that Voorhoeve had previou noted a similarity in distribution, appearance and disappearance of these substances in a wide variety of tissues.

The general upshot of all this work was to suggest some kind of causal c nection between glycogen-loss and the invagination process. We have alre remarked upon the curious flask-shaped cells concerned with invagination (p. 1

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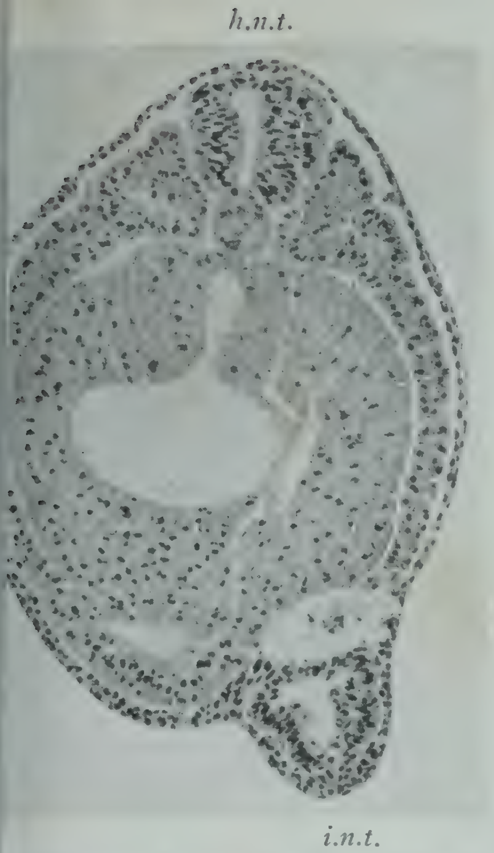
* From a slide kindly given me by Jean Brachet.



Induction of secondary neural tube by
antation of ether extract of glycogen.

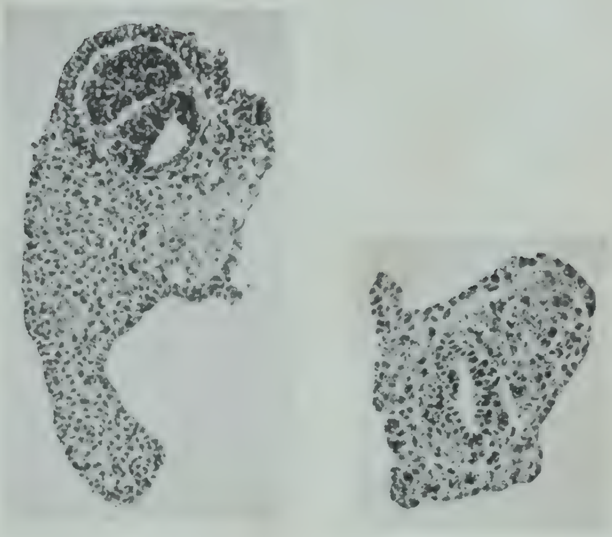
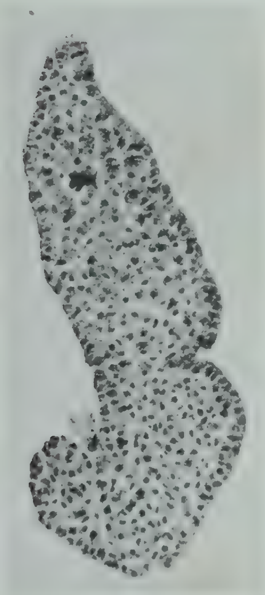


Fig. 101. Effect of methylene blue on explanted competent ectoderm; induction of neural tube; two cases.



100. Effect of methylene blue
explanted competent ectoderm;
ntrol in Holtfreter solution only.

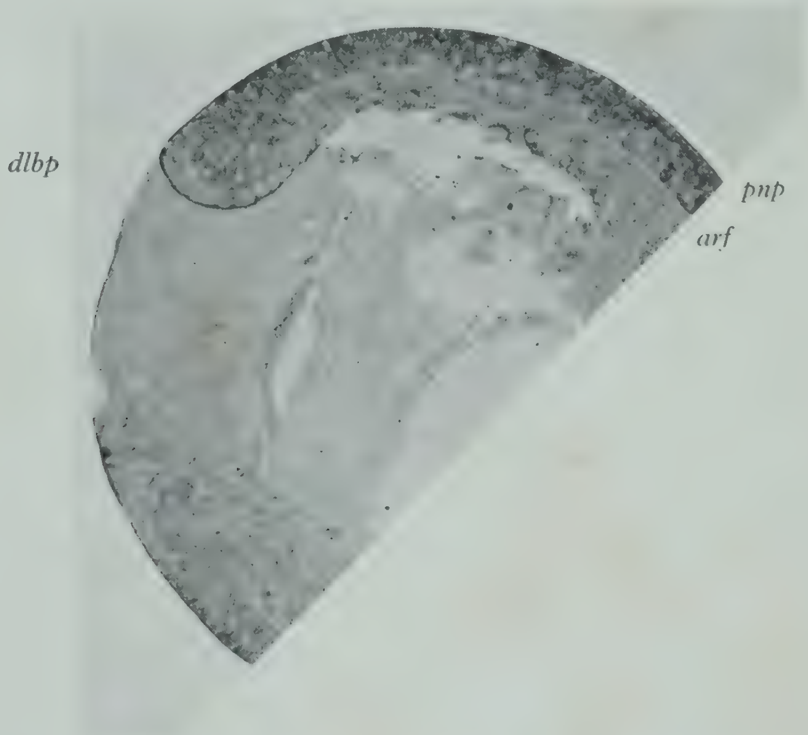


Fig. 102. Sharp disappearance of glycogen from the dorsal lip during invagination (the "*Glykogen-grenze*"). *pnp*, presumptive neural plate; *arf*, roof of the archenteron; ***dlbp*, dorsal lip of the blastopore.**

he impression of contractility which they have made upon several observers.
d there be something more than a coincidence in this association of glycogen
kdown and contractility in gastrulation?

reat though the pioneer value of histochemical work may be, it is particularly
erable to technical criticism. Such was the case with the glycogen boundary,
asteels & Léonard and Pasteels[4] brought serious objections of this kind against
onclusions of Woerdeman and Raven, who in the meantime had been largely
rmed by Tanaka, working in Japan on *Rana nigromaculata*,* and by Brachet[14]
iscoglossus. It was held that the loss of glycogen from the archenteron roof
an artefact due to the fixation fluids employed, and that this did not occur
dioxan method was used. We need not, however, enter into the details of
discussion, for the subject was put on a firm foundation by Heatley[1] and his
borators, using a direct chemical
hod. It thus turned out that though
scepticism of Pasteels had been justi-
e, Woerdeman was right.

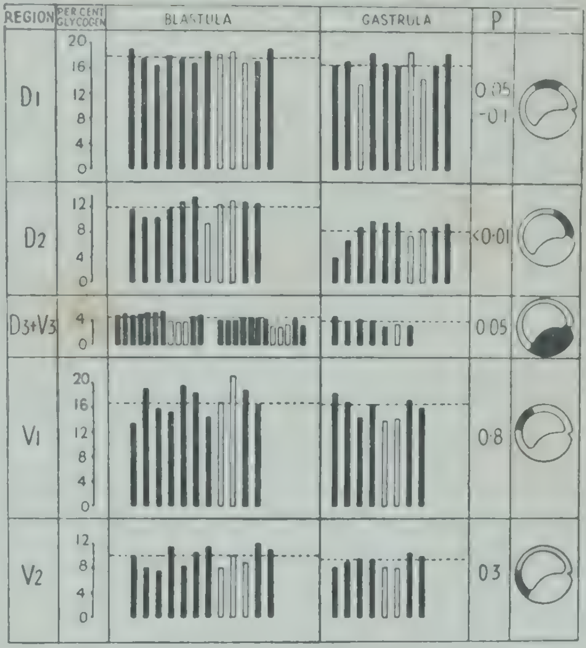


Fig. 103. Microchemical data demonstrating the marked fall in glycogen content of the invaginating dorsal lip of the blastopore.

eatley[1] adopted the micro-chemical
hods brought to a high level of per-
on by the school of Linderstrøm-
g at Copenhagen, and worked out
rocedure for the determination of
ogen in amounts of tissue of the
er of 1 mgm. with a probable error of
γ glycogen, using at the end of the
cess the thiosulphate micro-titration
hod of Linderstrøm-Lang & Holter[1]
reducing sugars. By these means
ley demonstrated a fall of about
in the dorsal lip of the blastopore
g invagination.† In a later paper
tley & Lindahl) this effect was con-
ed and a careful survey of all the
ns of the embryo showed that the fall of glycogen was inappreciable in
ns of the gastrula other than the invaginating material. Fig. 103 shows a
mary of their results.

e must therefore accept the existence of a fall in glycogen-content of the
ginating material coincident with gastrulation. Whether this occurs in other
s beside the amphibia will be discussed later (p. 334).

hat a general decrease occurs in the glycogen-content of the amphibian
ryo between the early cleavage stages and the time of hatching had long been
wn (*CE*, p. 1043), but in work parallel with that of Heatley's it was con-

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iyajima's paper contributes nothing here.

is work cannot be passed by without a mention that it was the first in which a direct and
le chemical method was applied to the various separated regions of a gastrula; hence a land-
in chemical embryology.

firmed by Brachet & Needham on *Rana temporaria* and by Takamatsu[2] o Japanese newt, *Hynobius nebulosus*. Since the work of Willstätter & Rohde it had been known that glycogen exists in cells and tissues in two forms, glycogen", readily extractable with boiling water or dilute trichloracetic and "desmo-glycogen", a further fraction only obtainable by breaking dow proteins with concentrated potash in the classical Pflüger method. Brach Needham[2] therefore investigated the amphibian embryo from this point of thinking it possible that the results would be of interest in relation to the ma in which the evocator is bound in the cells. With the Good-Kramer-Son micro-method the following figures were obtained:

	Mgm./40 embryos total glycogen	Lyo-glycogen % of total
Just fertilised eggs	3·24	91·1
Early gastrulae	3·25	93·2
Early neurulae	3·03	95·1
Advanced neurulae (closing neural folds)	2·69	97·0
Larvae at hatching	2·26	97·4

Thus the glycogen bound to proteins in the desmo-form, i.e. extremely tig always formed a very small proportion of the total glycogen. But during deve ment it decreased from about 9 % of the total glycogen to about 2½ %, while total glycogen (in % dry wt.) decreased from 16·7 to 10·7 %. It was interes that the fall at the beginning of neurulation (i.e. from the half-moon yolk- stage to the visible demarcation of the neural folds) was almost entirely accoun for by diminution of the desmo-glycogen.

Heatley & Lindahl also occupied themselves with determinations of lyo- desmo-glycogen. They discovered the interesting fact that at gastrulation desmo-glycogen decreases to a considerable degree in all parts of the emb not only in the dorsal lip of the blastopore. Their results are shown in accompanying table:

Table 17. *Glycogen in the amphibian embryo*

	Total glycogen			Desmo-glycogen				
Region	Blastula	Gastrula	Decrease %	Blastula	% of total	Gastrula	% of total	De
Presumptive neural plate	17·8	16·5	7	0·90	5	0·48	3	
Organiser region	12·0	8·3	31	0·50	4	0·32	4	
Yolk endoderm	4·3	3·9	9	0·32	7·5	0·24	6	
Presumptive epidermis	16·7	16·5	1	0·76	4·5	0·44	2·5	
Ventral ectoderm	10·0	9·3	7	0·57	5·5	0·46	5	

Thus in all the regions of the gastrula there is an absolute, and in most of th a relative, decrease in desmo-glycogen. Moreover, in the presumptive mesode which invaginates, the glycogen which disappears is not mainly desmo-glycog for the total decrease is nearly 4 mgm. %, while that for desmo-glycogen is than 0·2 mgm. %. It is evident, therefore, that desmo-glycogen cannot be only fraction of glycogen to which the evocator is attached in the cells if s an attachment exists.

his conclusion was entirely substantiated by the results of the implantation of no-glycogen and lyo-glycogen into the blastocoele cavity of the intact embryo leatley, Waddington & Needham. Both these fractions possess the power of ıction, i.e. contain the ether-soluble active material in adsorbed condition; from a statistical point of view, neither was more active than the other.

·352. Respiration and glycolysis in the regions of the gastrula

or a long time it had been evident that, side by side with direct estimation ıbstances in presumptive neural plate, archenteron roof, and ventral ectoderm, rvey of the characteristics of these tissues was necessary along the lines of Warburg technique, measuring the respiration, fermentation, and respiratory tient. But the technical difficulties in measuring accurately the gas exchange piece of tissue weighing, perhaps, only 50γ dry wt., were very considerable, in the earlier experiments probably not overcome.

he first investigation was that of Brachet[8]. He compared the respiration *ana temporaria* gastrulae, in which the dorsal lip had been destroyed by ery, with others in which a part of the ventral ectoderm had been similarly royed, using Fenn micro-respirometers and a small type of Warburg manoer. It resulted that the loss of the dorsal lip region entailed a considerably ter diminution of oxygen consumption than the loss of ventral ectoderm. ıparisons of the carbon-dioxide elimination by the Saunders pH indicator hod on isolated pieces of dorsal lip and ventral ectoderm gave similar results, al lip : ventral ectoderm; 1·89 : 1·00). The experiments could, however, only egarded as qualitative, since no measurement of the size of the pieces was e.* In a second series of experiments, Brachet[10] modified the Saunders hod to include a Linderstrøm-Lang titration, and measured the amount of solated pieces of *Discoglossus pinctus* gastrulae by a usual micro-Kjeldahl od giving the total nitrogen. Again the carbon-dioxide elimination was 84 % r in the case of the dorsal lip than in that of the ventral ectoderm. He also estimations of respiratory quotient by the Meyerhof-Schmitt method, ning 1·1 for the dorsal lip and 0·77 for the ventral ectoderm. At the same Waddington, Needham & Brachet measured the oxygen uptake of *Triton tris* isolates from dorsal lip and ventral ectoderm in a modified Gerardline micro-respirometer, the dry weight of the pieces being obtained on a o-balance. In this case the oxygen consumption for unit weight proved to f exactly the same order in the two cases. Brachet & Shapiro devised a very nious apparatus in which the intact gastrula was made the centre of two ard-Hartline capillaries, so that the respiratory rate of one hemisphere could ompared directly with that of the other (Fig. 104). Using embryos of *Rana atica* a difference of 47 % was found in favour of the dorsal lip hemisphere. ı later paper, however (Brachet[22]), where *Rana fusca* was used, the rence was hardly detectable for oxygen though clearly seen for CO_2 (29 %), ı indicating an R.Q. difference.

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r the same reason we must disregard the later experiments of Stefanelli[3], though he used a d technique.

Finally, Fischer & Hartwig[2] attacked the problem by the more straightfo but rather prosaic method of using Warburg manometers with cups of rela small volume, and a large number (sometimes as many as sixty) isolates from dorsal lip and ventral ectoderm. Even then the observed excursions were not very far from the thermobarometric range, and the experiments had to be continued for as many as 24 hr. Dry weights were obtained directly. Under these conditions dorsal lip region showed an oxygen uptake not more than 20 % higher than ventral ectoderm. The significance of this difference is questionable.

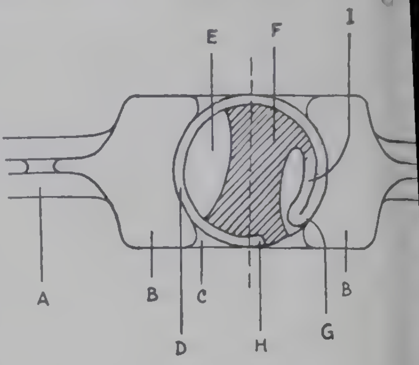

Fig. 104. Brachet-Shapiro apparat measuring Q_{O_2} of the intact amph gastrula. *A*, *A*, two Gerard-Ha capillary manometers; *B*, *B*, m meter chamber; *C*, gelatin t the gastrula; *D*, ventral ecto *E*, blastocoele; *F*, yolk endod *G*, dorsal lip of blastopore; *H*, ve lip of blastopore; *I*, archenteron

The first essential, therefore, if manometric experiments on the regions of the amphibian gastrula were to be carried through with some conviction of reliability, was an ultramicro-manometer 1500 to 2000 times more delicate than the standard Warburg apparatus. This need was met in the summer of 1 by the suggestion that the Cartesian diver should be employed for the purp

The use of the Cartesian diver as an ultramicro-manometer was first propo by Linderstrøm-Lang[2], and further details of the method as used by the Cop hagen school were given in a later communication (Linderstrøm-Lang & Glic The Danish workers, however, did not apply the method for use with min pieces of tissue. It was adapted for the problem of the gastrula by Needha Boell & Rogers. At the same time an ultramicro-Kjeldahl method capable accurately determining between 1 and 20γ protein N was worked out by Needha & Boell and employed for measuring the amounts of tissue used in the dive As will be seen, the Cartesian diver* micromanometer proved of the utmost val for the range of gas exchanges required.

If a bubble of air or gas is enclosed in a small glass vessel so that it floats with a larger vessel, the buoyancy of the diver will vary according to the pressu imposed on the whole system, and it will sink or rise as this pressure rises falls. If the pressure is approximately constant, the diver will hover in unsta equilibrium. Conversely, if the gas phase in such a diver is increased or diminish in amount by the process of some chemical reaction inside it, the pressure requir to maintain it at a given level will correspondingly rise or fall. In this way t diver is equivalent to a constant volume manometer if from time to time it brought to a zero line and the pressure required to do so noted.

The principle of the method is shown in Fig. 105. For different purpo different arrangements of the divers were used. B shows a diver of the simpl type, used for the measurement of anaerobic glycolysis. The tissue and t physiological medium occupy the bottom of the diver bulb, beneath which th is a glass tail to maintain the diver in a vertical position. The amount of glass

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* It appears that the Cartesian diver has nothing to do with Descartes. It was first descri by Raffaele Magiotti, a pupil of Galileo, in a little book on the incompressibility of water (16 which he dedicated to Lorenzo di Medici. The book contains a diagram of the diver, which wa doubt later called "Cartesian" as a synonym for anything "scientific" or mechanical, and wh remained without application, a philosophical toy, until the present time.

tail is adjusted so that the equilibrium pressure of the diver is fairly near that
ne atmosphere. The neck is partly occupied by a drop of oil, so placed that
e is a small bubble of air between the oil and the flotation medium. The
ition medium, a strong salt solution giving minimum gas solubility without
ssive viscosity (lithium chloride, ρ 1·245), is seen within the diver vessel in
105 A, with the diver floating in it. The diver vessel is submerged in an

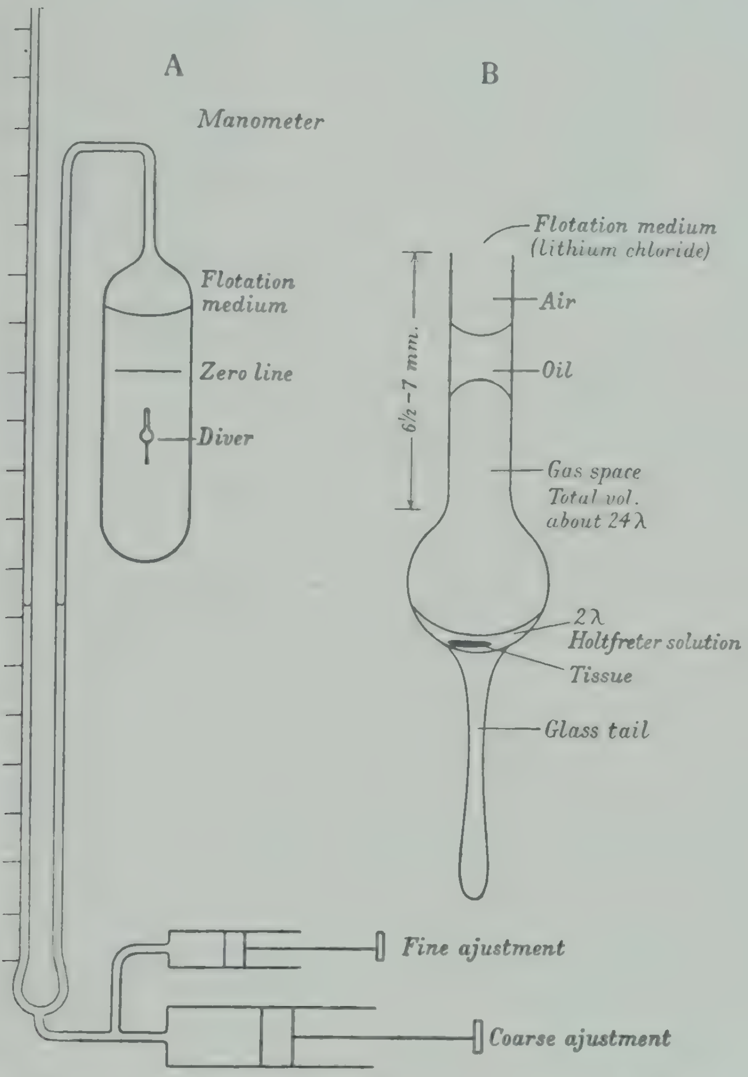
A
B
Manometer
Flotation medium
Zero line
Diver
Fine ajustment
Coarse ajustment
Flotation medium
(lithium chloride)
Air
Oil
6½–7 mm.
Gas space
Total vol.
about 24λ
2λ
Holtfreter solution
Tissue
Glass tail

Fig. 105. A. Principle of the Cartesian diver manometer. B. Diver arranged for measurement of $Q_{\text{L}}^{\text{N}_2}$.

irately regulated thermostatic water-bath, and connected to a water mano-
er, the pressure in which can be adjusted (as in a Warburg manometer) by a
nge mechanism. It is convenient to have fine and coarse adjustments. Readings
accurate to 0·5 mm. and it is therefore possible to measure changes of the
er of a millionth of a cubic centimetre of gas. To take a reading, it is only
essary to bring the diver to a temporary standstill at the zero line, and to note
pressure.

or measurements of processes other than anaerobic glycolysis, divers variously
lified were used. Fig. 106 A shows a diver arranged for measurement of oxygen
sumption (Boell & Needham); the neck is waxed and supports beside the

oil-seal a drop of alkali to absorb CO_2. B shows a diver arranged for the meas ment of respiratory quotient (Boell, Koch & Needham). The neck is somev longer than before and waxed throughout its length, it supports a column of as well as the oil-seal and the alkali. During the first part of the experim CO_2 is absorbed by the alkali and the diver's buoyancy steadily falls, but at conclusion of the respiratory period an excess pressure of about 12 in. Hg is up in the diver vessel by means of an accessory manometer. This forces the al and acid together down into the bulb and so liberates the absorbed CO_2. As

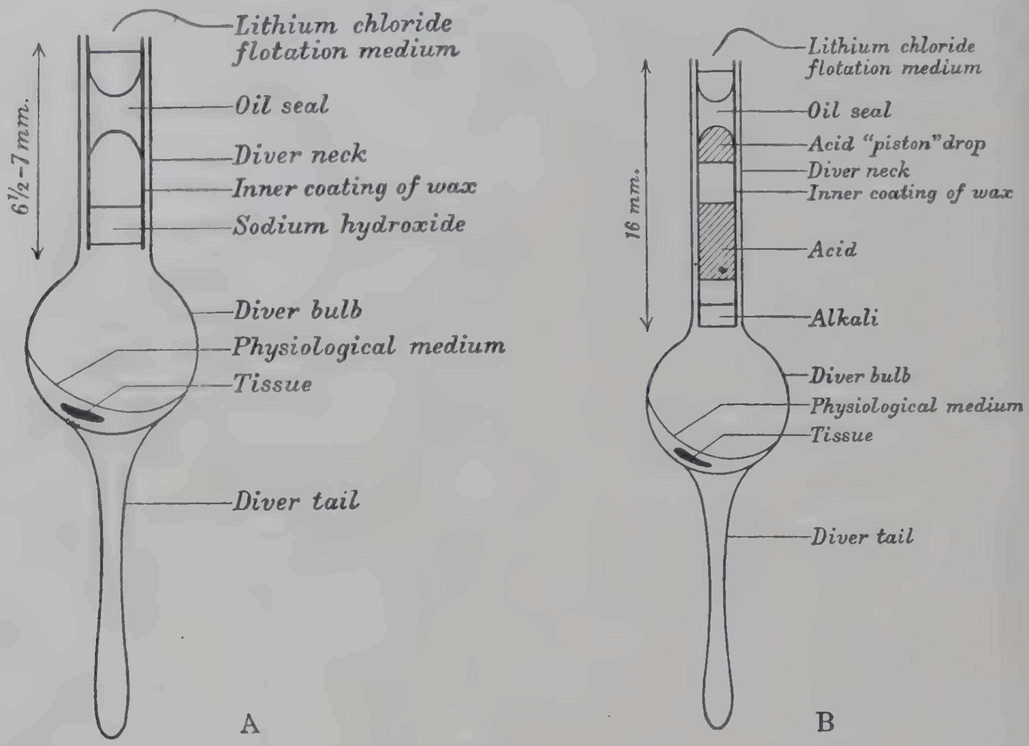
Lithium chloride flotation medium
6½–7 mm.
Oil seal
Diver neck
Inner coating of wax
Sodium hydroxide
Diver bulb
Physiological medium
Tissue
Diver tail
A
Lithium chloride flotation medium
16 mm.
Oil seal
Acid "piston" drop
Diver neck
Inner coating of wax
Acid
Alkali
Diver bulb
Physiological medium
Tissue
Diver tail
B

Fig. 106. A. Diver arranged for measurement of Q_{O_2}. B. Diver arranged for measurement of R.Q.

oil-seal returns exactly to its original position when the excess pressure is releas the liberated CO_2 can be easily measured. The principle of the method is t of Dickens & Šimer[1]. Finally, a pair of divers can be arranged for the measu ment of aerobic glycolysis according to the "double cubical cup" method Warburg (Needham, Rogers & Shen). One diver contains much more s pension liquid and less gas phase than the other, hence, owing to the high solubi of CO_2, less of this gas from respiration or aerobic glycolysis enters the gas ph than in the diver with little liquid. From the behaviour of the two divers aerobic glycolysis may be calculated.

Details of the Cartesian diver methods,* which will probably be of great h in all branches of biological investigation where minute structures have to examined, will be found in the papers mentioned. The fermentation of o yeast gives an easily measurable long-continuing linear change and the respi tory quotient of a dorsal lip can be determined on an overall gas turnover of 0·1 The gas change most commonly used in the following measurements of gastr regions was of the order of 200–300 mμl. in 2 or 3 hr. from pieces of tissue conta ing from 5 to 15γ protein nitrogen.

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* In what follows ml. = cubic centimetre; μl. = cubic millimetre = λ; mμl. = c.mm. × 10^{-3}

he relation of the Cartesian diver method to other micro-manometric methods best be appreciated by comparing them in the form of a table (modified from ı), as follows:

Table 18. *Comparison of micro-manometers*

Type of manometer	μl. gas change per cm. on the manometer scale	Nearest unit to which meniscus can be read	Approximate sensitivity per such unit, μl.
dard Warburg (cf. M. Dixon)	20	0·5 mm.	0·5–1·0
l Warburg, as used for metabolism issue cultures (Laser[1]; Meier)	3–7	0·5 mm.	0·15–0·35
o-manometers of Fenn (Schmitt; yee; Fischer & Hartwig[2])	3–5	0·1 mm.	0·03–0·05
o-manometer of Krogh (adapted n Bodine & Orr)	2	0·2 mm.	0·04
llary micro-manometer (Howland ernstein)	1	10 μ	0·001
llary micro-manometer (Gerard & tline; Brachet & Shapiro; ddington, Needham & Brachet) losed chamber	1·8–2·3	6 μ	0·001
rate chamber micro-manometer n mica mirrors optically read atley[2]; Heatley, Chain & enblum)	0·04–0·08	—	—
fine capillary micro-manometer fanelli[1])	0·06–0·12	0·5 mm.	0·003–0·006
o-differential of Fenn type hmitt)	5	1 μ	0·0005
esian diver manometer (Linder-m-Lang[2]; Boell, Needham & gers)	0·003–0·022	0·5 mm.	0·00015–0·001

he actual working range of the diver, as of any respirometer, should of course o chosen that the approximate sensitivity shown in the table forms only a ll part of the differences regarded as significant.

ith gastrulae of *Rana temporaria* Boell, Needham & Rogers obtained the wing figures for anaerobic glycolysis:

	$Q_L^{N_2}$	NH_3 production ($\mu l.10^{-3}/\gamma$ dry wt./5 hr.)
Dorsal lip region	+0·63	2·31
Ventral ectoderm	+0·21	0·97

nother series of experiments on *Triton alpestris* gave a similar, though not e so large, difference. It was thus clear that the anaerobic glycolytic rate is ıt three times as high in the dorsal lip of the blastopore as it is in the ventral derm. This substantial difference appeared also in the anaerobic ammonia duction. There was no difference between the regions in the ratio between ompensated and ammonia-compensated carbon-dioxide output. Some nonia measurements were done on other stages also; they showed what ears to be a rise in ammonia production at the beginning of the action of the nary organiser followed by a fall (see p. 624).

wing to the replacement of inert yolk by active protoplasm, tadpole tissues wed a $Q_L^{N_2}$ in the neighbourhood of + 3·0.

The action of dinitro-*o*-cresol on the anaerobic glycolysis of the regions studied by Boell & Needham[1] in a separate paper. It had little effect on ammonia production but raised the uncompensated CO_2 production so 300 %; the $Q_L^{N_2}$, therefore, which depends on both factors, was raised an intermediate degree. The effect, however, was exerted to a much grea extent upon the ventral ectoderm than upon the dorsal lip region; in the case the uncompensated CO_2 production, indeed, the ventral ectoderm activity w raised to the level of that of the dorsal lip. This difference was compared Boell & Needham[1] with that between the Q_{O_2} of diapause and developing gra hopper embryos, and that between Q_{O_2} of unfertilised and fertilised echinoder eggs.

The former was studied by Bodine & Boell[2] in the course of a series of investig tions on the developing orthopteran embryo, details of which will be found Section 3·32. As is well known, the grasshopper embryo develops actively first for a few weeks but then enters a state of dormancy, the diapause, in whic no morphological development occurs. This lasts several months, after which period of intense activity, both morphological and metabolic, leads in three wee to the end of embryonic development. Respiratory rate is considerable durin the two active periods, but sinks to a low steady state during the diapause. Sinc the respiration in the two active periods is powerfully inhibited by KCN and CO while that of the diapause is not, it is to be supposed that diapause respiratio proceeds mainly by "non-ferrous", possibly flavin-catalysed, systems. It wa now found that taking normal respiration as 100, the diapause respiration wa raised by nitrophenols to 375, while that of the active periods only to 250. Th extra respiration was cyanide-sensitive. The fact that the respiratory quotien was raised from the usual diapause level of 0·75 to unity was at first attributed t the extra respiration being at the expense of carbohydrate but it was not fluoride- or iodoacetate-sensitive, and ammonia estimations demonstrated that at leas three-quarters of it was due to protein breakdown.

Table 19. *Changes produced in metabolism of amphibian gastrula regions by treatment with dinitro-*o*-cresol*

		Dorsal lip region	Ventral ectoderm
$Q_L^{N_2}$:			
	Normal	0·63	0·21
	Dinitro-*o*-cresol	0·73	0·54
	Difference	+0·10	+0·33
	% rise	16	157
Uncompensated CO_2 ($\mu l. 10^{-3}/\gamma/5$ hr.):			
	Normal	0·82	0·28
	Dinitro-*o*-cresol	1·11	1·07
	Difference	+0·29	+0·79
	% rise	35	282
Ammonia production ($\mu l. 10^{-3}/\gamma/5$ hr.):			
	Normal	2·31	0·97
	Dinitro-*o*-cresol	2·23	1·62
	Difference	−0·08	+0·65
	% change	−3	+67

In the case of the amphibian gastrula, however, the fact that dinitrocresol did not raise the ammonia production to any extent suggests that here the action is ly if not entirely upon carbohydrate utilisation. This agrees with the findings onzoni & Ehrenfest on mammalian muscle.

he parallel with the sea-urchin egg is of much the same kind. The respiratory hanisms here involved will be discussed on pp. 563 ff., but it is sure from work of Runnström[7] and others, that the respiration of the unfertilised egg ot sensitive to cyanide, while that of the fertilised egg, proceeding at a much er rate, is so. Later, Runnström[14] found that although pyocyanin, added accessory hydrogen transporter, increased the respiratory rate of both un- lised and fertilised eggs, its effect was of the order of 200 % on the former only 80 % on the latter. The effects of nitrophenols on the sea-urchin embryo been studied by Clowes and his collaborators (see Krahl & Clowes[1,2] for the ature). Most of their attention was devoted to the interesting fact, discovered hem, that concentrations of nitrophenols and halophenols which give the imal stimulation to respiration also cause a reversible blockage in the hanism of cell-division. In one place, however (Clowes & Krahl[1]), they state the effect of nitrophenols on respiration was greater for the unfertilised for the fertilised eggs—a six times against a four times increase. Thus e the respiration of the fertilised egg is normally four times that of the rtilised egg, after both had been treated with dinitrophenol, it was only two a half times as great.

n entirely different type of reagent, dimethyl-*p*-phenylene diamine, was found unnström[10] to raise both unfertilised and fertilised egg respiration to the same level, indicating that the low activity of the unfertilised egg is not due to any ciency in the indophenol oxidase.

ll these phenomena resemble to some extent the state of affairs found for the hibian gastrula regions. In general we may say that wide variations in abolic activity exist in correlation with various physiological and morpho- etic states. The regions or states of high activity may or may not be in a dition of maximum possible activity, but the regions or states of low activity certainly damped down in some way or other. For respiration, this damped e may be due to a block in the chain of carriers in the Warburg-Keilin system, hat such respiratory activity as there is has to proceed by way of a "non- us" system. For glycolysis we can now begin to visualise the damping anism since it has been shown that the glycolytic enzymes are themselves tive to oxidation (see the reviews of Rapkine[9] and K. C. Dixon). In the case e amphibian gastrula, at any rate, the fact that the organiser substance, or ator, is liberated in a region of high activity, and not in a region physiologically ped, can hardly be without significance.

xygen consumption of dorsal lip and ventral ectoderm was investigated by l & Needham[2], using the gastrulae of *Discoglossus pictus* and *Amblystoma canum*. In neither case could any appreciable difference between the respiratory of dorsal lip and that of ventral ectoderm be observed.

	Q'_{O_2} (related to total N, not dry weight)	
	Discoglossus	*Amblystoma*
Blastula roof	—	−2·63
Dorsal lip region of gastrula	−4·80	−3·21
Ventral ectoderm of gastrula	−4·93	−3·18
Closing neural folds	−3·02	

The measurements in the paper of Waddington, Needham & Brachet were confirmed rather than those of Brachet & Shapiro. We may nevertheless a the view that the difference between the two hemispheres of the gastrula f by Brachet & Shapiro in their double-capillary apparatus is real. But sinc gastrula is a relatively complicated morphological structure rather than a h geneous ball, this only means that there is more of the rapidly respiring tiss one hemisphere than in the other. It does not mean that the dorsal lip r has a higher intrinsic respiratory rate than the ventral ectoderm. Direct meas ments show that the two rates are statistically identical.

Later work by Boell, Nicholas & Sawyer confirmed the above observations established the existence of zones of respiratory rate, ranging from a maximu anterior presumptive neural plate and anterior presumptive epidermis, thr intermediate values for posterior presumptive epidermis and dorsal lip regio a minimum in the yolk-plug. This may explain some of the differences betw the results of different investigators noted above. The exact region take compare with the dorsal lip may have varied a good deal. Intrinsic respira rate, however, is probably very similar throughout the gastrula, since di centrifugation measurements of the amount of yolk in the various regi show an inverse proportionality between yolk-content and respiratory rate.

As a good deal of work has been done on the respiration of intact amphib gastrulae, it is of interest to compare the figures in the literature with those fou by the diver method on isolated regions. The following table summarises the

Table 20. *Respiration of intact amphibian gastrulae*

	$T°$	μl. O_2 uptake/ 100 embryos/hr.	μl. CO_2 outpu 100 embryos/
Białaszewicz & Blędowski:			
Rana temporaria, gastrulae	20	24·5	—
Saunders:			
Rana temporaria, gastrula	14	—	24·4
Parnas & Krasinska:			
Rana temporaria, gastrulae	—	13·7–25·0	—
Wills:			
Amblystoma punctatum, Harrison stage 10, gastrulae	25	34	—
Triturus torosus, Harrison stage 10, gastrulae	25	39	—
Rana pipiens, Harrison stage 10, gastrulae	25	39	—
Stefanelli[1,2]:			
Rana fusca, cleavage stages	?	(9–18) av. 13	—
Brachet & Shapiro:			
Rana sylvatica, gastrulae;	25		
Dorsal lip hemisphere		70·6	—
Ventral ectoderm hemisphere		48·1	—
Brachet[6]:			
Rana temporaria, gastrulae	?	29·9	—

If now we compare these results with those reported by Boell & Needham[2] find that the respiration of dorsal lip region and ventral ectoderm is a good d higher than would be expected from the volume of embryo occupied by the From the data on the axolotl gastrula, the average amount of protein N tak was 11·1γ or $\frac{1}{32}$nd part of the total protein nitrogen in the embryo. As $\underset{\sim}{Q}'_{O_2}$ is the order of 3, the oxygen consumption for the whole embryo would

l. 100 embryos hr., a figure considerably higher than any given in the table. ould, however, be remarked that in Brachet & Shapiro's measurements, the e of 70µl. 100 embryos hr. was also much above the average for the whole t embryo. We can certainly conclude that the yolk endoderm must have an emely low respiratory rate and occupies a large proportion of the total weight nitrogen of the embryo.

is interesting to compare the absolute levels of Q_{O_2} reported by Boell & dham[2] for the amphibian embryo with those for the mammalian egg. For the unfertilised egg of the cow, Dragoiu, Benetato & Opreanu have found values ging from −21 to −35. The contrast with those of the order of −0·5 or under he amphibian embryo remains great even when we allow for the 17° difference mperature, and must doubtless be due to the fact that the amphibian egg is ly lecithic, and that much yolk is present even in cells deriving from the nal pole (see footnote on p. 157).

he same consideration applies to comparisons between the respiratory rate astrula regions and adult amphibian tissues. Thus tadpole epidermis, rding to Erdmann & Schmerl and Börnstein & Klee, has Q_{O_2} of from −1·21 −2·18 (cf. the $Q_{L}^{N_2}$ figures reported by Boell, Needham & Rogers for tadpole lermis of −1 to −2). This contrasts with the −0·2 to −0·3 for ventral derm (Boell & Needham[2]). Börnstein & Klee found that skin from old frogs a lowered respiratory rate (though not to the gastrula level) and that explants kin had a much higher Q_{O_2}, up to −6·75. Comparable data for adult nervous e will be found in Winterstein & Hirschberg and Gerard.

n connection with the respiratory rate of the presumptive mesoderm and ral tissue it is interesting that Brachet[21] found neural differentiation can go on M/1000 KCN but not in M/3000 iodoacetate.

here still remains, however, the observation of Brachet[10,22] that dorsal lip ates showed a greater CO_2 elimination than ventral ectoderm pieces. This could mean, as Brachet himself had found, a difference between the two regions spiratory quotient. When the respiratory quotient of the two regions was stigated by the new methods in the work of Boell, Koch & Needham, this ctation was confirmed. The dorsal lip of the blastopore shows a much greater l towards unity than the ventral ectoderm. The figures are given in the mpanying table and in Fig. 107:

	Gastrulae of *Amblystoma mexicanum*
	R.Q.
Blastula roof	0·75
Half-moon yolk-plug gastrula:	
Dorsal lip region	1·0
Ventral ectoderm	0·83
Three-quarter-moon yolk-plug gastrula:	
Dorsal lip region	1·0
Ventral ectoderm	0·89
Closing neural folds of young neurula	1·0

s as soon as gastrulation begins the dorsal lip region attains a respiratory tient of unity. The respiratory quotient of the ventral ectoderm also rises, more slowly, and has not exceeded 0·90 by the time when further dissection of pure ectoderm is practically impossible owing to the expansion of the und lying mesoderm.

The exact significance of these results must be a matter for further resear We may wish to assume that the respiratory quotient of unity of the dorsal during gastrulation implies a predominantly carbohydrate catabolism and associate this both with the breakdown of glycogen there shown by Woerdem and Heatley and with the liberation of the primary organiser. But we m remember that protein breakdown ending in ammonia may also give a respirat quotient approximately unity. The quotients of the ventral ectoderm certai give the impression of a mixed fat-protein catabolism. At all events it is clea that we must see a change in *quality* of metabolism, rather than in *quantity*, setting in at the organisation centre at the onset of gastrulation.*

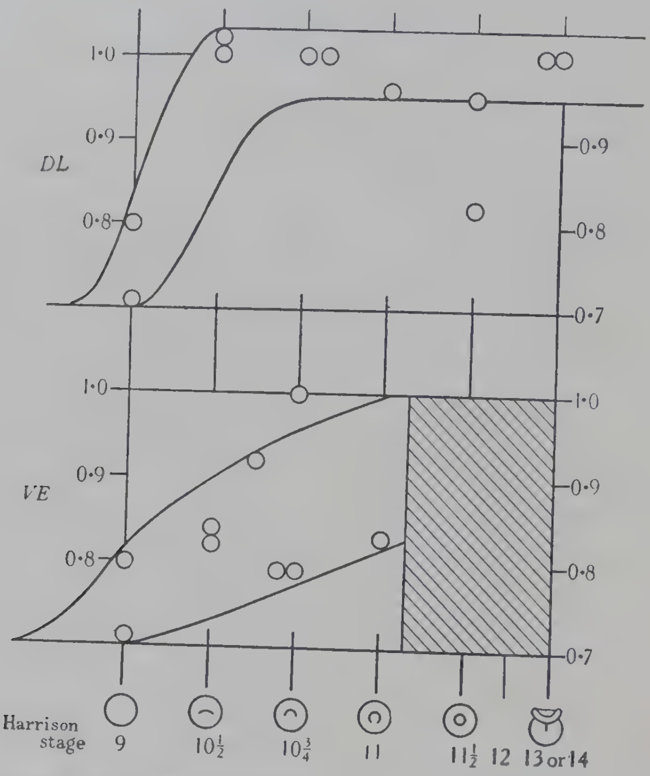

Fig. 107. Respiratory quotient of the regions of the amphibian gastrula. *DL* dorsal lip of th blastopore; *VE*, ventral ectoderm.

Finally, the aerobic glycolysis was measured by Needham, Rogers & Shen, and although the double-cup method, whether on the standard or the ultramicro scale, does not give results of such accuracy as the other manometric methods, it was established that there is little or no aerobic glycolysis in either of the two regions of the gastrula. The Pasteur effect is thus equally efficient throughout the gastrula.

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* In connection with the differential metabolism of the regions of the gastrula, it will be remembered that according to Bellamy and Bellamy & Child, disintegration begins in the dorsal lip of the blastopore when embryos are treated with toxic concentrations of mercuric chloride, potassium cyanide, and ammonia. The claim was contested by Cannon, and the original papers must be referred to by those who wish to estimate its significance. The dorsal lip of the blastopore has, of course, long been claimed by Child to be a centre of "high metabolism".

umming up then, we find as between dorsal lip region and ventral ectoderm
arked difference in anaerobic glycolysis and anaerobic ammonia production,
difference at all in respiratory rate or aerobic glycolysis, and considerable
erences in respiratory quotient. The way is now evidently open for much
her extension of our knowledge of the metabolism of the gastrula, a knowledge
ch alone will enable us to understand the mechanism of liberation of the
ary evocator from its inactive precursor.

353. The role of sulphydryl in gastrular metabolism

he foregoing results may be set beside some interesting work done by Fischer
artwig[1] and by Piepho[1], in which it was found that when gastrulae are stained
various vital dyes and then placed under strictly anaerobic conditions, there
more rapid reduction of the dye in the dorsal lip region, and later in the
ral plate, than elsewhere. This difference was seen not only in the intact egg
also between isolates from the regions. Though it is never possible to be very
ise regarding the meaning of the intra-cellular reduction of coloured indicators,
e results must surely indicate a greater activity of certain oxido-reduction
ems in the organisation centre relative to the other parts of the embryo.*
f considerably more importance is the study of the fixed –SH groups in the
rula suggested by Rapkine[8] and carried out in interesting work by J. Brachet[18].
mbryos of the unpigmented *Triton cristatus* are used it is possible to ascertain
distribution of fixed sulphydryl by performing the nitroprusside reaction
he whole embryo after denaturation of the proteins with trichloracetic acid.
is is done on the oocyte the large nucleus, but not the cytoplasm, is coloured
ep pink.† At the time of fertilisation the pink colour is seen only in a zone
he animal pole; Brachet[18] supposes that the proteins containing fixed –SH
ps have been derived from the nucleo-plasm during maturation. Yolk-
elets never give the reaction. Two hours after fertilisation the grey crescent,
he animal pole, shows the colour when the test is performed, and later on
greater part of the blastula roof gives the reaction. After the appearance of
lastopore, the organisation centre shows it intensely. It is present over the
e presumptive neural plate area and intensified at the cerebral end. In the
ula all the neural area shows it.

nce the total area which colours bright pink increases from fertilisation to
end of neurulation, there would seem to be a synthesis of proteins containing
h fixed –SH at the expense of the yolk-proteins.‡ Brachet[18] then noted other
rences between the proteins in different gastrula regions. The distribution
ranules staining red with the pyronine-methyl-green method was exactly
lar to that of the fixed –SH groups. This was not at first understood,
Brachet[24] was later able to show, using a nuclease specific for ribo-nucleotides,
the pyronine-staining, basophilic, material is probably cytoplasmic (phyto-)

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the results by similar methods on the chick (p. 335) and on echinoderms (p. 496).

igh fixed –SH content has been observed also in the nucleus of the echinoderm oocyte by
etto and by Ries[2] (see pp. 136, 567), and in that of the amoeba also by Chalkley[2] (see p. 422).

the former somewhat similar observations of Chatton, Lvov & Rapkine on ciliates.

nucleoprotein (see Sections 3·434 and 3·44). This linkage of the fixed – protein in the organiser region with the cytoplasmic nucleotides may well b the greatest importance. We are reminded of the high inductor activity of nucl material (Waddington[19], cf. p. 171) and of the presence of phyto-nucleotides embryonic and sarcomatous tissue (Claude[2], cf. p. 269). Indeed, it may be coincidence that the latter are normally combined with plasmalogen, a substa known to disappear from the invaginating material during gastrulation (Vo p. 189 above).

Centrifugation experiments of Pasteels[11] strengthen the view that the fixed –S proteins have something to do with the organiser effect. If eggs in the cleava stages are centrifuged as thoroughly as may be without rendering cleava abnormal, the fixed –SH proteins are thrown to the animal pole with the fa layer away from the yolk and pigment. At a later stage, though a blastopore m form, gastrulation will not progress farther; in this case it is found that t arrested embryo will show no fixed –SH proteins at the dorsal lip region. the normal induction may be inhibited partly or wholly (see p. 226); in this ca no fixed –SH reaction will be obtainable from the inhibited regions.*

It may be added that glutathione and cysteine showed no inducing power the *Einsteckung* experiments of Finkelstein & Schapiro[1], but the abov mentioned work of Brachet[18,24] applies to fixed, not free, –SH groups, and t phenomena in normal development are probably very complex. We cannot yet fit this into its proper place.

In any case, the careful study of inhibitors on gastrulation will be very illu minating, especially where their chemical action is already fairly definitely know Iodoacetate and fluoride have been used by J. Brachet[21,22,23]. Fluoride in doses high as $M/40$ (sufficient to inhibit glycolysis completely in the chick embryo) ha no effect on amphibian development before hatching (we can hardly doubt that penetrates into the egg) and weak concentrations of iodoacetate ($M/300$) hav also no effect, though these would be expected to inhibit glycolysis. On the othe hand $M/30$–$M/60$ iodoacetate, while allowing cleavage *and* gastrulation to procee normally, arrest development at the stage of the full-moon yolk-plug, i.e. ju before the appearance of the neural folds and during the induction process. Th inactive concentrations of fluoride and iodoacetate have no effect on the respiratio of the whole embryo, but the effective concentration of iodoacetate inhibits th respiration of the gastrula 90 %. Respiratory quotient (Meyerhof-Schmi method on thirty embryos) is lowered in all the inhibitors, whether morph genetically effective or not, to 0·83; lactic acid production and glycogen break down are greatly inhibited. If at this inhibited stage the embryos are replace in ordinary water, development will continue normally, but if they are allowe to remain in the inhibitor, dissolution will follow.

All this strengthens the suggestion that sulphydryl groups have something to d with neural induction. It may be, of course, that there is a reversible denaturatio of the protein in the inactive precursor complex. Or the oxidation-reductio ilibrium may be affected. The latter possibility is supported by the work of chet & Rapkine (only available as yet in preliminary form) who could provoke ral differentiation in explants of ventral ectoderm by immersing them in icing solutions (–SH; $M/10$ reduced glutathione, $M/10$ thiomalic acid, 10 cystein) and could suppress neural differentiation in dorsal lip explants mmersing them in oxidising solutions (–S-S–; $M/20$ oxidised glutathione; 50 alloxan). Confirmation of this will be of much importance. We may recall the flavine-adenine compound which acts as the prosthetic group of the no-acid oxidase will only join up with the protein moiety of the enzyme n in the reduced condition.

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* Criticisms of the technique used in all this work, made by Giolitti, were successfully rebutte by J. Brachet[18].

n all further development we shall have to bear in mind three problems: the metabolic events which give rise to the active substance, (2) the nature of active substance itself, and (3) the metabolic events accompanying and ciated with the neuralisation response of the competent tissue.

·355. The nature of the inactive evocator complex

t has been suggested above that the hypothesis which explains most simply facts known about the distribution and function of the primary evocator is t it is normally inactivated by combination with other substances, probably tein, perhaps polysaccharide also. From this combination it is normally rated only in the dorsal lip of the blastopore. In other regions of the gastrula nay also be liberated by any treatment which will denature the proteins of the ue.* Some preliminary evidence exists which suggests that the primary cator is a substance of steroid nature. Interest therefore attaches to the nplexes ("symplexes" or "cénapses") between proteins and lipins which have n more thoroughly studied. In adult tissues the primary evocator is found the free form, but from the first formation of the oocyte till gastrulation it is d in combination, and thus competent tissues which would otherwise react to e protected from its influence until their competence is lost.

reatment less severe than denaturation of the proteins may apparently liberate evocator. Such an activation carried out by dyes has been described above 189); the dye may act by displacing the evocator from its combination. The actions obtained by nucleic acid (see above, p. 180) might receive a similar lanation since Stenhagen & Teorell have shown that nucleic acid will displace rubin from its association with serum albumen.

he association which we have seen to exist (see p. 190) between the nary evocator and glycogen suggests a further extension of the foregoing othesis. Is the inactivating complex one in which glycogen is involved as well protein? In this case a metabolic attack either on the protein or on the glycogen ght break up the complex and liberate the evocator. That the complex cannot

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n his recent *Silliman Lectures*, Spemann ([17], p. 244) seems to maintain that the appearance of evocator in the ventral ectoderm after treatment with heat cannot be the same process as the earance of the evocator in the dorsal lip of the blastopore during normal metabolic events. this distinction, however, there are no convincing reasons. The fact that a loose complex be broken down to liberate an active substance in many different ways renders superfluous a ef in the existence of more than one such loose complex.

be identical with desmo-glycogen was shown by the experiments describe p. 193, but the evocator might be associated with another fraction of intra-cel glycogen.

That loose complexes exist in the cell has been known since the time of Ho Seyler (1866); perhaps lecitho-vitellin of egg-yolk is the most classical exam Without preliminary treatment with alcohol it is impossible even to approa complete separation between the protein and the lipin. The monograph Macheboeuf and the reviews of Przyłęcki[2,3] give an account of these comple The phenomenon of "lipo-phanerosis" (see Macheboeuf and Lison), also long been known to cytologists; under certain treatments there may great increase in the amount of lipid histologically detectable. A particul striking instance is due to Heilbrunn. If echinoderm eggs are treated ammonium salts in fairly high concentration and then centrifuged, a la apparent increase in the fatty layer is observed (see Fig. 108), indeed the hyal zone may be reduced to a mere streak. An analogous effect was obtained amoebae with ultraviolet irradiation by Heilbrunn & Daugherty.

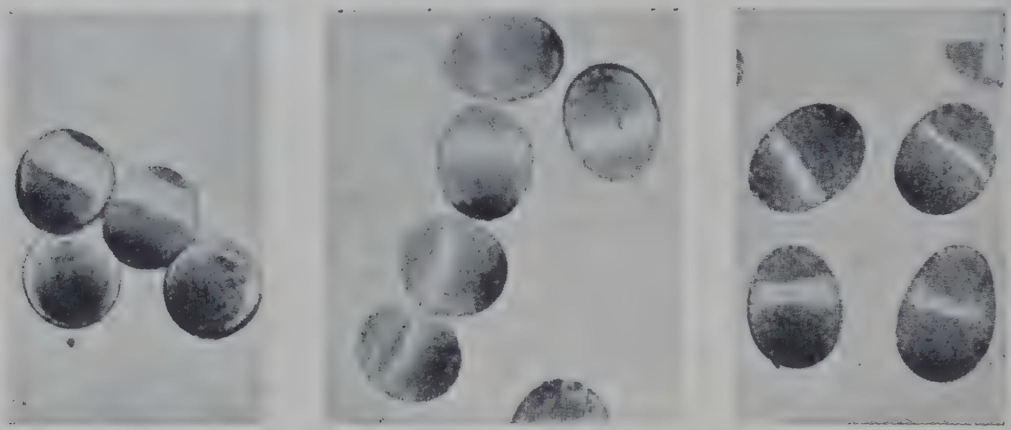

Fig. 108. Lipo-phanerosis in echinoderm eggs demonstrated by centrifuging.

Further evidence to our purpose may be adduced from the literature as follo

Complexes of proteins with polysaccharides

Lyo- and desmo-glycogen (Przyłęcki & Białek; Willstätter & Rohdewal Przyłęcki[3]; Gurfinkel)

Myosin and glycogen (Przyłęcki & Majmin[1]; Mystkowski[1]; Mystkowski, Stil & Zysman)

Polysaccharides and clupein (Przyłęcki, Gedroyć & Rafałowska)

Starch, dextrin, and various proteins (Przyłęcki & Rafałowska; Rafałows Krasnodebski & Mystkowski; Przyłęcki, Andrzejewski & Mystkows Przyłęcki & Dobrowolska; Przyłęcki & Grynberg[1]; Bartuszek; Przyłęcki Targońska)

Studies on the nature of the linkage (Przyłęcki, Kasprzyk & Rafałows Przyłęcki, Cichocka & Rafalowska; Przyłęcki, Rafałowska & Cichoc Gedroyć, Cichocka & Mystkowski)

Complexes of proteins with unsaponifiable lipins

Sterol-protein combinations in blood (Gardner & Gainsborough; Brug Mellander; Bills; Theorell)

ol-protein combinations in yeast (McCorquodale, Steenbock & Adkins)
ol-protein combinations in wool (Schoenheimer & Breusch)
icial sterol-protein complexes (Przyłęcki, Hofer & Frajberger-Grynberg) in surface films (Rideal & Schulman).
genic steroid-protein substances (Marrack)
ochrome-protein complexes (Palmer & Eckles; Kühn & Lederer; Wald)

Complexes of proteins with saponifiable lipins

tho-vitellin
od-lipins (Macheboeuf & Sandor; Wu, Liu & Chen; B. Delage; Grigaut)
ficial protein-fat complexes (Przyłęcki & Hofer). The freer a protein is from fatty material the more rapidly is it attacked by proteases (Janicki; Przyłęcki[3]). Mixed lipo-protein monolayers have been studied by Schulman; Schulman & Hughes; Schulman, Stenhagen & Rideal.

Complexes of saponifiable lipins with polysaccharides

y acids and corn starch (Taylor & Nelson; Taylor & Sherman)
ithin and glycogen (Przyłęcki & Majmin[2])
in phosphorus and starch (Posternak)

Complexes of unsaponifiable lipins with polysaccharides

ty acids and phytosterol on banana starch (Lehrman & Kabat)

Complexes of unsaponifiable lipins

rols and carcinogenic hydrocarbons (Clowes, Davis & Krahl)

Complexes of nitrogenous substances with proteins

cleic acid and serum proteins (Przyłęcki & Frajberger; Przyłęcki & Grynberg[2])
otine and proteins (Leontiev & Alexandrovsky)
c acid and proteins (Przyłęcki, Grynberg & Szrajber; Przyłęcki & Kisiel; Grynberg & Kisiel)

he literature on these complexes is thus a large one, and it is by no means ned that the above-mentioned papers, or those referred to later in this ion, constitute a full list of all the complexes which have been studied. Much ertainty as to their nature exists. A complex may be a well-defined chemical npound with a stoichiometric relation between its components, it may be a ociable adsorption complex, or it may merely be the co-existence of two or re substances which the methods hitherto available have failed to separate. us cataphoresis has proved to be the only means of separating polysaccharide m flavoprotein. Modern methods of protein chemistry tend more and more ead away from the vague views of earlier colloid chemistry, and to throw light the nature of the linkage in the true complexes. As yet, of course, we have rdly any knowledge of the extent to which physiological significance depends on the nature of the linkage in the complex. At all events no need for hesitation ists in picturing the unmasking or activation of the primary evocator as a eration of it from inactive combination.

Throughout the field of biochemistry it is becoming more and more clear that properties of compounds are very greatly dependent on whether they exist free, or in more or less loose combination with other molecules, such as pro of which they may be prosthetic groups, or polysaccharides, with which may form complexes. All the logical possibilities seem to be represe Thus:

(1) A compound may be "active", or possess a certain property, both i bound and the free state. In this case,

(1 *a*) the same activity may be shown, or

(1 *b*) the nature of the activity may be quite different.

(2) Activity may only be manifested in the bound state.

(3) Activity may only be manifested in the free state.

Examples of (1 *a*) are the proteolytic enzymes, some of which, such as pe and kathepsin, are believed (Willstätter & Rohdewald[1]) to occur in two fo such as desmo-kathepsin, strongly attached to the protein cell structure, lyo-kathepsin, much more readily extractable. The enzymic activity is, howe the same in both cases (Bamann & Salzer).* Some drugs, moreover, appe enter into close combination with proteins, e.g. the trypanocidal Bayer (Boursnell & Wormall).

Examples of (1 *b*) are very common. Thus lactoflavin, in the free state, is v min B_2, but when attached to protein (as ribitylflavin-phosphate), forms the called "yellow" enzyme or co-enzyme dehydrogenase of oxidation-reduct processes (cf. Karrer's review[2] and Fig. 307). It seems that the strict line of demar tion between proteins with well-defined prosthetic groups and proteins united w other substances as complexes is breaking down. Warburg[4] has proposed the v that co-enzymes are, in fact, the prosthetic groups of their corresponding enzym Thus adenosine triphosphate, which we may represent as adenine-ribo phosphate-phosphate-phosphate, is the classical phosphorus-transporter in phosphorylation cycles of glycolysis (see Fig. 322). But co-enzyme I (co-zyma which may be written adenine-ribose-phosphate-phosphate-ribose-nicotinic ac is the co-enzyme, and hence the prosthetic group (when the active complex been formed), of the triose-phosphate dehydrogenase. Co-enzyme II differs o in the possession of a third phosphoric acid group, and would be regarded as prosthetic group of another series of dehydrogenases. The co-enzyme of amino-acid oxidase from a structural point of view unites the fields of glycoly fermentation and oxidation, for its structure is adenine-ribose-phospha phosphate-ribitol-flavin. Another instance is vitamin B_1 (Aneurin or Thiam United with two phosphate groups it forms co-carboxylase, presumably prosthetic group of the carboxylase of yeast. Baumann & Stare, in their rev on co-enzymes, make the interesting suggestion that in the body thiam pyrophosphate is united with ribose and adenine. This would bring all co-enzymes into one group. These ideas are extended to pigments in a review K. G. Stern[2]. Finally, it is known that vitamin B_6 (Pyridoxin or Adermin; rat anti-dermatitis vitamin) occurs in a protein-bound form, though it has same activity whether bound or free (Kühn & Wendt).

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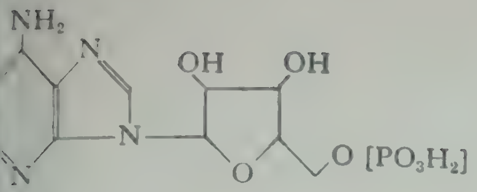
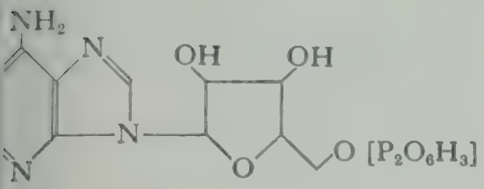
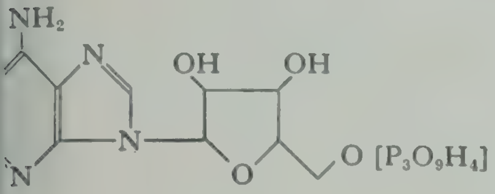
* We shall see later (p. 610) a case of some importance for embryology in which a w system of enzymes appears to be of the desmo-type, namely that concerned with the "r phosphorylating" glucolysis; in contrast to the enzyme systems of the phosphorylation cy of glycolysis which have long been available for study in tissue extracts.



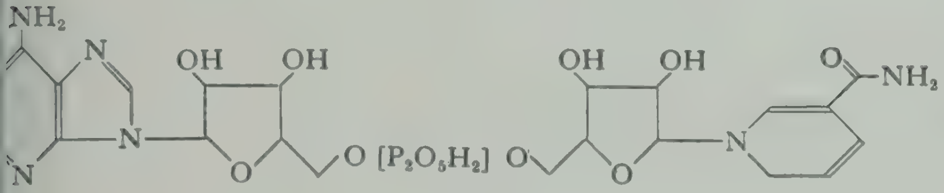
Adenosine monophosphoric acid
(Muscle adenylic acid)



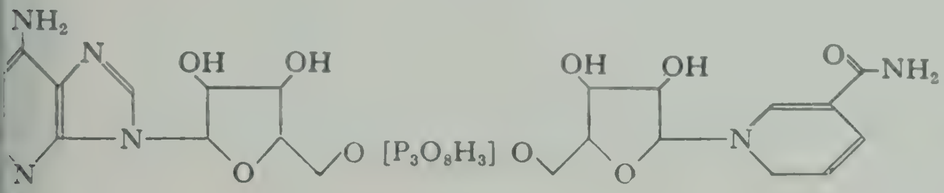
Adenosine diphosphoric acid



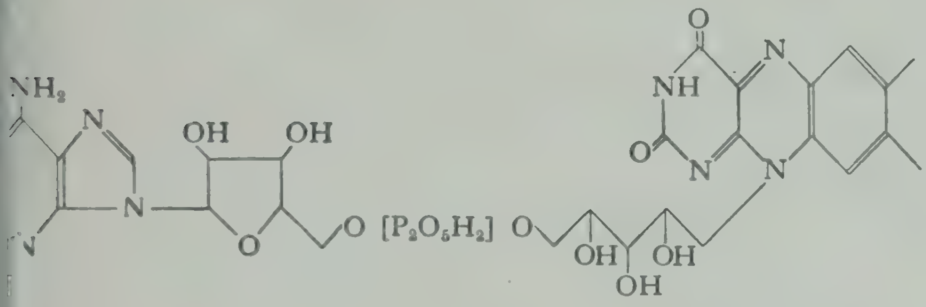
Adenosinetriphosphoric acid



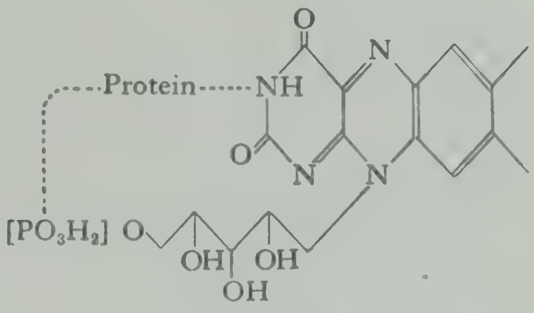
Cozymase
(Coenzyme I)



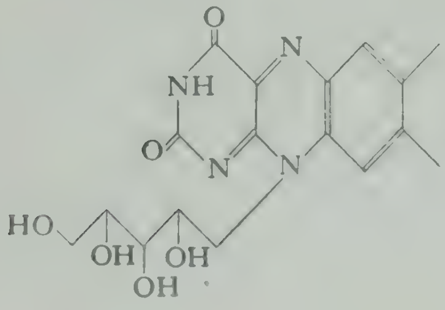
Coenzyme II



Amino-acid
oxidase coenzyme

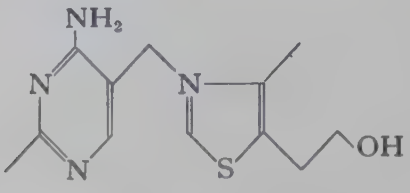


Flavoprotein
("Yellow enzyme")

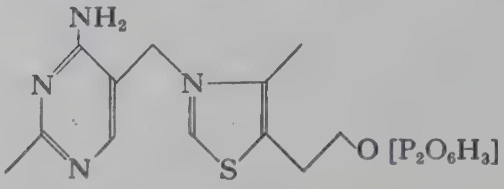


Lactoflavin
(vitamin B_2)

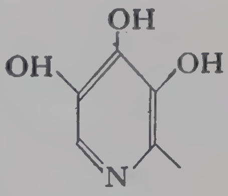
On the other hand, there are many examples of substances which are act *only* in the combined form (case (2) above). While such compounds as haema have little biological activity, the tetrapyrrol system, once combined with prote gives such powerfully active substances as cytochrome, catalase, peroxidase, t



Aneurin (vitamin B_1)



Co-carboxylase



Pyridoxin (vitamin B_6)

haemoglobins, etc. (Keilin[3]).* Leaf chlorophyll is also said to be in protein co bination (E. L. Smith). In the same way, vitamin A, a lipochrome (see p. 24 only carries on the reversible changes characteristic of the retina when it has be combined with a protein as visual purple in the form of the pigment retine (Wald; Wald & Clark). Retinene is set free when visual purple is treated w chloroform or otherwise denatured, and the colour changes from purple greenish-yellow.

There is no reason why two pigment molecules should not be attached to t same protein, e.g. the photosynthin of certain bacteria (French), in which bo bacteriochlorophyll and a carotenoid appear to be contained.

Curiously enough, a carotenoid system related to visual purple occurs in eg Yamamoto[3] has described an interesting lemon-yellow pigment in the eggs o polychaete worm, *Ceratocephale osawai*. On strong illumination the colo changes to green, probably because the lipochrome-protein complex is dissociate and in the dark the process is reversed. Both processes can go on anaerobica and do not affect the oxygen consumption of the eggs.

Properties such as colour are indeed often dependent on complex-formatio Another lipochrome, that of crustacean (e.g. lobster) eggs and carapaces, astac (see p. 249), is green when in combination with protein, but pink when fr (Kühn & Lederer; Kühn, Lederer & Deutsch; Fabre & Lederer; N. A. Sørensen Any denaturing agent will liberate the lipochrome, and if we regard the pink for for the sake of argument as "active" we have a model for the liberation of t vocator. Stern & Salomon demonstrated the interesting fact that the liberation f the pigment from its protein shows mild thermal reversibility (at temperatures ot above 70°). Still more striking was the observation of Peters & Wakelin that ·aces of soaps (such as sodium oleate, palmitate, tetradecoate and dodecoate) ·ould act like alcohol or chloroform as liberating agents in concentrations as ›w as 1×10^{-3} M. The effect was not seen with fatty acids below C_{12}, i.e. below ıe change from water- to "fat"-solubility. The liberated pigment can be put ack on to its protein by the action of 0·03 M $CaCl_2$. It is possible that we have ere an explanation of the neural inductions caused by fatty acids in the experi-ıents of Fischer *et al.* (see p. 180) as it is unlikely that fatty acids would ›ng remain wholly in the free form within, or in close contact with, living ssues.

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* The open tetrapyrrol system, though biologically inactive, also combines with protein, e bilirubin-serum-albumen (Pedersen & Waldenström).

† The bound form has been called "ovoverdin" but this seems a rather unfortunate name, sin the term "uteroverdin" has already been used by Lemberg[1] for an open-chain tetrapyrrol deri tive, found in the mammalian placenta, closely related to the blue pigment of birds' egg-sh (see p. 647) and identified with biliverdin. It used to be thought that astacin was still unit with a fatty acid when in the "free" state, but Kühn & Sørensen have shown that the presum saponification was an oxidation converting astaxanthin to astacin. Lipochrome-protein comple also occur in insects (Abeloos & Toumanov).

We thus approach group (3), the substances only active in free form, which ıost closely resemble the position of the evocator in the amphibian gastrula. 'he plant growth hormone, auxin (see p. 266), which also in other ways shows ıteresting parallels with the evocator, is known to exist in inactive, bound, form Kögl *et al.*; Allen; Went & Thimann). There is also evidence that vitamin C scorbic acid) exists in bound form combined with protein in plant tissues Guha & Pal; Reedman & McHenry).[*] Human blood, according to Sinclair, ›ntains a good deal of vitamin B_1 in bound, inactive form, and the same ay possibly be true of milk (Houston & Kon). There is also a bound form vitamin E (Emerson, Lu & Emerson). Acetylcholine, liberated suddenly at ıolinergic nerve-endings (cf. the review of G. L. Brown), appears to arise om a pharmacologically inactive non-dialysable and denaturable protein com-ex precursor, according to Loewi; Kahane & Levy; H. C. Chang, and Mann, ennenbaum & Quastel.[†] Sympathin, too, liberated at adrenergic nerve-endings, ust exist there in some stored form (cf. the review of Rosenblueth), and adrenalin-:otein complexes are being investigated (Utevsky & Levantzeva; Utevsky & inskaia). And the water-soluble heat-stable melanophore-controlling crust-:an neuro-humors (Parker) seem also to increase in activity after boiling, as if erated from protein complexes.

We are thus coming to the general conception of combination with protein as : most important element in biological activity. An "active" substance of low ›lecular weight will generally require combination with a specific protein in ler that its activity may be manifested.[‡] And it may be held in inactivated ·m on another, perhaps equally specific, protein. It may well be that the evocator s to be adsorbed on to a protein in order to carry out its effects, just as it has be previously liberated from inactive combination. Unfortunately it is as yet

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But cf. Fujita & Ebihara.

There are those, however, e.g. Trethewie, who do not accept the evidence so far advanced for ›ound" acetylcholine, but they seem to be fighting a rearguard action.

It is, of course, essential to specify exactly what activity is in question. Thus flavin-adenine-ıucleotide united with one protein will form the amino-acid oxidase; united with another it l form a co-enzyme dehydrogenase. To some extent the tools of the cell seem to involve :rchangeable parts; several ends may fit on to one handle or several different sorts of handle to one end.

extremely hard to picture exactly how the effect of the primary evocator produced in the competent cells. This problem will be referred to again Section 3·5.

2·356. The evocator compared with plant hormones

We spoke above of auxin. This well-known plant hormone has been the subje of a large amount of research, ably summarised in the monographs of Boyse Jensen[1] and of Went & Thimann.* Auxin, formed or liberated at one part of plant (e.g. the tip of the *Avena* coleoptile), is transported from cell to cell an stimulates longitudinal growth of the cellulose walls at another. Its effects a therefore entirely different from those of the primary evocator in animal develo ment; one stimulatory substance being concerned with growth, the other wit differentiation. Nevertheless the parallels between the action of auxin an evocator are so interesting, and the differences so instructive, that it is wort while to give them a brief discussion.†

In the first place, it is evident that what we have previously spoken of competence enters into plant physiology also, as it probably does in any syste of stimulus-response reactions. Thus the stem, but not the root, is stimulate by auxin, and there is indeed a reversal of reactivity, for the elongation of th root is inhibited by auxin (W. & T. pp. 14, 213) though the number of roots increased (W. & T. pp. 183 ff.). Similarly, in the *Avena* coleoptile, the productio of auxin and the growth rate at first increase side by side, but later on the growt rate falls off some considerable time before the auxin production shows any sign of decreasing (W. & T. p. 59). Competence to grow therefore fails before th supply of stimulatory substance.

We may next distinguish five points in which auxin and evocator action ar analogous, and two points in which they seem to differ.

(1) Bound auxin. By no means all the auxin in a plant is present in the fre state (B.J. pp. 58, 63; W. & T. pp. 64, 65, 68, 116, 132). Three states of auxi are defined: the storage form, set free by saponification of, e.g., seed oils, and henc probably an ester; the "bound" form, extractable only by organic solvents suc as chloroform; and thirdly, the free form, capable of diffusing out of the pla into an agar block placed in intimate contact with a cut surface. In germinatio

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* Referred to in what follows as B.J. and W. & T. respectively. All the literature will be foun in these monographs. There are also the valuable reviews of Kögl; Went; Avery; Boysen-Jensen Thimann[1]; Thimann & Bonner; Haagen-Smit; Erxleben.

† There is also the question of the existence or action of auxin in animal embryos. Agar impre nated with auxin, when implanted into the blastocoele cavity of amphibian embryos, produce only the usual ectodermal thickenings (du Pan & Ramseyer). On the growth of embryoni fibroblasts it has no marked effects (Ramseyer & du Pan). But it is certain that auxin (or heter auxin) is synthesised during embryonic development. In the amphibian embryo there is no before gastrulation, but it increases rapidly after the closure of the neural folds (Rose & Berrier It is also definitely synthesised by blowfly and silkworm moth embryos (Berrier). In the chic embryo, as a careful paper by Robinson & Woodside shows, there is a relatively enormous increa of it during development.

he coleoptile grows from a minute bud on the mesocotyl to a length of 4 cm., vith few or no cell-divisions. Auxin is continuously produced by the growing ip, which itself does not elongate, and there is to all appearances a close parallel iere between the liberation of auxin in free form at the tip and the liberation of he evocator in the dorsal lip of the blastopore. When cut off and placed on agar, he tip continues to produce auxin for many hours, but ceases to do so at last, robably on account of exhaustion of the precursor. When estimation methods s quantitative as those for auxin have been worked out for the evocator, we hall be able to follow similarly the production of free evocator. Normally he tip receives supplies of the precursor from the seed.

(2) Species-specificity. Like the evocator, auxin effects show no differences etween species and genera of the plants used as source of stimulus or reactant B.J. p. 57; W. & T. p. 114).

(3) Just as we have seen reason to believe that a variety of substances may nmask the evocator in the reacting tissue, so certain unspecific influences have een demonstrated to produce their effects by way of the auxin mechanism in lants (B.J. p. 114; W. & T. pp. 130, 191). To this class of effect belong the rowth stimuli of acids and of ethylene.

(4) Irradiation with X-rays may inhibit the growth of coleoptiles, but application of auxin later shows that this is not due to interference with the competence B.J. p. 66). These facts may be compared with those to be discussed shortly egarding the evocator (see p. 222).

(5) The auxin effect involves no change whatever in respiratory rate (B.J. . 122; W. & T. pp. 128, 164), though the growth process is dependent on espiration in that it will not go on anaerobically and that cyanide inhibits it *ari passu* with respiration. On the other hand, dinitrophenol accelerates respiraon but not growth. Here again there is a striking similarity with the differentiation ormone of the gastrula.

The apparent differences between auxin and evocator action are equally teresting. Thus:

(1) Auxin is destroyed by the action of oxidising enzymes at cut surfaces V. & T. p. 57), or in extracts.

(2) Auxin disappears in the plant metabolically (W. & T. p. 86). In the same ay acetylcholine after its action in the adult vertebrate body is destroyed by oline esterase and adrenalin by adrenalin oxidase. On the other hand, there is information available about the destruction of the evocator. Homoiogenetic duction (p. 154) shows that as the neural tube is determined, so its cells acquire duction power, and in later development the evocator appears free in all tissues. he question of its disappearance will not be solved till adequate estimation ethods become available for it.

In the universality of its distribution, the primary evocator resembles another lant-growth hormone, or rather, group of hormones, the Bios complex. In 1901 Vildiers, working on a suggestion of M. Ide, established that yeast cells will not row and divide normally if the amount in which they are first sown is too small. ubsequent research (well summarised by Kögl & Tönnis) demonstrated the presence of the missing necessary substances in wort,* and Bios was eventuall split up into three synergistic, not summative, factors. Bios I is now known be *i*-inositol (Eastcott).† Bios II (sometimes called biotin) has been crystallise by Kögl & Tönnis and prepared by them from hen's egg yolk and white by process involving esterification, distillation of the volatile ester, and hydrolysi This product is active in a concentration of 1 in 4×10^{11}. It has been shown b Kögl & v. Hasselt to be present in all animal tissues, and is perhaps identic with "vitamin H" and "co-enzyme R" (György *et al.*). A similar substance wa studied by R. J. Williams and his collaborators and named by them "Pantothen acid". This substance, now known to be a dipeptide of α-hydroxy-β-β-dimethy γ-hydroxybutyric acid and β-alanine (Williams & Major; Williams, Finkelstei Folkers, Harris, *et al.*), also occurs in all animal, and some plant, tissues an seems to be present in some protein-bound, non-diffusible form in egg-whi (Williams, Lyman, Goodyear, Truesdail & Holaday). Purified by brucine, a pr paration has been obtained active at 1 part in 2 billion of culture mediu (Williams, Truesdail, Weinstock, Rohrmann, Lyman & McBurney). It has a effect on yeast respiration as well as on yeast growth, stimulating the Q_{O_2} of yea 60 % (Pratt & Williams). It seems to be identical with the chick anti-dermatit factor (B_3) of Jukes. Bios III is the least well understood of the factors in th group, but Kögl & Tönnis have established that it contains nitrogen though n sulphur or phosphorus.

These facts are mentioned in order to show that cases of highly active substance widely, if not universally, distributed among living tissues, are already known.

2·357. Induction and fertilisation

Before leaving the discussion of phenomena which may be parallels of em bryonic induction, a word must be said about fertilisation. The question whethe induction is in any sense related to echinoderm parthenogenesis is a very legitima one. Unfortunately, the subject of the parthenogenesis of the eggs of invertebrat is at present in a state of some confusion, for apart from the reviews of J. Loeb and Y. Delage (in 1909 and 1910 respectively) nothing is available except that Just[2] which upholds the "fertilisin" theory of F. R. Lillie[5,7]‡ in a rather partisa way. A detailed account of the literature in this field from a modern point of vie is greatly needed.

Space is not available here to embark upon a discussion of even the mos important relevant facts (see F. R. Lillie & Just), but it is certain that echinoder eggs give off a number of active substances into the surrounding water. Thi "egg-secretion" is not produced by immature eggs, and is no longer produce y fertilised or parthenogenetically stimulated eggs. It can be washed out by epeated changes of water, thus rendering the eggs unfertilisable, but their ertilisability can be restored, as Woodward showed, by adding further egg-ecretion. During the fertilisable period, a wide variety of agents, including all inds of chemical substances (Y. Delage), blood or tissue juices (J. Loeb; Bogucki[2]), nd changes in osmotic pressure and temperature (R. S. Lillie[1]), will bring about arthenogenesis. Of these the most effective in common use is hypertonic sea-ater (double the normal salinity). Loeb's view[3] was that all parthenogenetic gents caused a cytolysis of the superficial layer of the egg, and hence the "release f a catalyser"; and for this view there is still much to be said. In its most odern form, suspicion has fallen upon calcium, the presence of which either utside or inside the egg seems proving to be essential for all types of parthenoenesis (Pasteels[1]; Mazia[2]; Heilbrunn & Wilbur). Other agents would simply ffect the threshold to or competence for the action of calcium.

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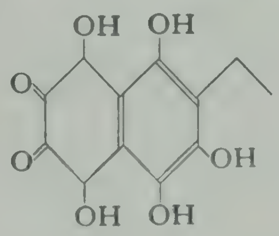
* W. Lash Miller, one of the earlier investigators of the Bios factors, met certain criticisms the work as follows: "All those who say 'no doubt some chemical in wort makes all the differenc but it need not be Bios', have qualified to join the Last Ditch Bacon Club, which holds th Shakespeare's plays were written, not by Shakespeare, but by some other person bearing th same name." This is not without a moral for embryologists.

† Other cycloses are inactive (Kögl & v. Hasselt).

‡ To me, it seems that F. R. Lillie's interpretation of fertilisation along immunological lines ha done more harm than good. This mistake would be interesting methodologically as showin that one should not use concepts taken from a level of *unnecessarily* high complexity.

On the whole, it seems that artificial parthenogenesis of echinoderms has more common with the stimulation of a nerve than with embryonic induction, for e very wide range of successful stimulators always suggested a physical rather an a chemical mechanism of immediate response. The parallel with nerve was fact used by the earliest student of parthenogenesis, Tichomirov[2], who in 1886 iscovered the sulphuric acid parthenogenesis of silkworms. Parthenogenesis ith sperm extracts has never been achieved.

The active substances of the egg-secretion have not so far been fully analysed. here is (1) the activator for spawning of the male. This is believed to be glutaione or a similar sulphydryl compound in the case of polychaetes (Townsend), ut in molluscs its properties are different (Galtsov). There is (2) the activator or sperm motility. In echinoderms this seems to be a volatile substance, since eam distillates of egg-secretion are effective (Clowes & Bachman), as also ropyl, amyl, and cinnamyl alcohols. There is then (3) the substance responsible r the chemotactic attraction of the spermatozoa (sometimes called aggregation); is has been recently identified by Hartmann, Schartau, Kühn & Wallenfels as e egg-pigment echinochrome, a polyhydroxy-naphthoquinone; though the claim contested by Tyler[8]. There is finally (4) the substance or substances called



Echinochrome (1:2:3:4:5:6:8-heptaoxy-7-ethyl-naphthalene)

fertilisin" by F. R. Lillie[7]. This consists of two factors, the cleavage factor, in e absence of which development cannot proceed, even if a sperm should enetrate into the egg; and the agglutination factor, which causes the spermatozoa stick together around the egg-jelly. The fact that agglutination power and rtilisability often ran parallel (C. R. Moore; Just[1]) led Lillie to regard the two effects as due to one single substance, but it seems more likely that two a involved, since O. Glaser[4] and Woodward were able to separate them, t former being undialysable and precipitable with ammonium sulphate, the latt being dialysable and precipitable with barium chloride. Yet Carter found th both effects were obtainable with thyroxin, which restores fertilisability an also agglutinates spermatozoa. The whole subject urgently needs reinvestigati by up-to-date chemical methods, as in the new work of Tyler and Tyler & Fox

Parthenogenesis brought about by material obtained from other eggs wou be a parallel for induction performed by killed ventral ectoderm. This has be claimed to occur by O. Glaser[1] ("autoparthenogenesis"), but the experimen have never been generally accepted and still require confirmation.

Parthenogenesis, then, resembles nervous irritability rather more than embryon induction. In the last resort it may be, of course, that all cases of stimulati are ultimately identical, but nerve reaction differs from reaction to a hormone b its much greater unspecificity, probably because rapid physical changes play greater part in it than the enzymic changes which hormones must occasion.

We must now return to the gastrula and consider the biological aspect of th liberation of the evocator.

2·36. Blastopore formation and the liberation of the evocator

During the preceding discussions the reader must often have asked what it that governs the place of appearance of the blastopore and hence the liberatio of the evocator and the determination of the neural axis. To answer this questio we have to go back to a period of development which has received less attentio than gastrulation, namely that of the fertilised egg and the embryo during earl cleavage.

The dorsal lip of the blastopore and part of the presumptive neural plate are represent the dorsal surface of the future larva. In the Anura the site of the dors lip of the blastopore is foreshadowed in the fertilised but uncleaved egg by th so-called "grey crescent", a band of material lighter than the animal pole bu darker than the vegetal pole. A similar "dorso-marginal" zone exists in Urodel but not so obviously. Our problem concerns the origin and significance of th zone, and hence, since it distinguishes dorsal from ventral, the origin of th bilateral symmetry (dorso-ventral asymmetry) of the embryo. Experiment work on the subject has been very confused, but the position has recently bee clarified.

Everyone is agreed that in the fertilised egg there are two gradients,[†] th animal-vegetal one, obvious on account of the accumulation of yolk towards th

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[*] A word may be added concerning interesting recent work on copulation-stimulating substanc in algae (Moewus; Kühn, Moewus & Jerchel). The cells of *Chlamydomonas eugametos* produc in light of all wave-lengths, a substance which accelerates the movements necessary befo copulation. This is crocin, the digentiobiose glycoside of crocetin, a lipochrome, and it is said be effective in dilutions of 1 in $2{\cdot}5 \times 10^{12}$. Cis-crocetin-dimethyl-ester, in mixtures of vario proportions with trans-crocetin-dimethyl-ester, forms the male and female substances. The are effective in dilutions of 1 in 3×10^8.

[†] I use the word here with no "arrières pensées" or presuppositions about metabolic question

al pole and the corresponding reduced division rate there*; and the dorso- al one, with the grey crescent as its apex (see Fig. 109). It is generally held the animal-vegetal gradient originates from the position of the egg in the , receiving its reserve materials from a capillary vessel reaching one point only s surface. Bellamy's proof of this and its subsequent withdrawal are of the mportance because on general grounds such a mechanism may be taken as mely probable. But the origin of the other gradient, or, if you will, the ion of the grey crescent, has been difficult to determine. In 1883 Roux[1,4] ved that he had proved a strict correspondence between the point of entry

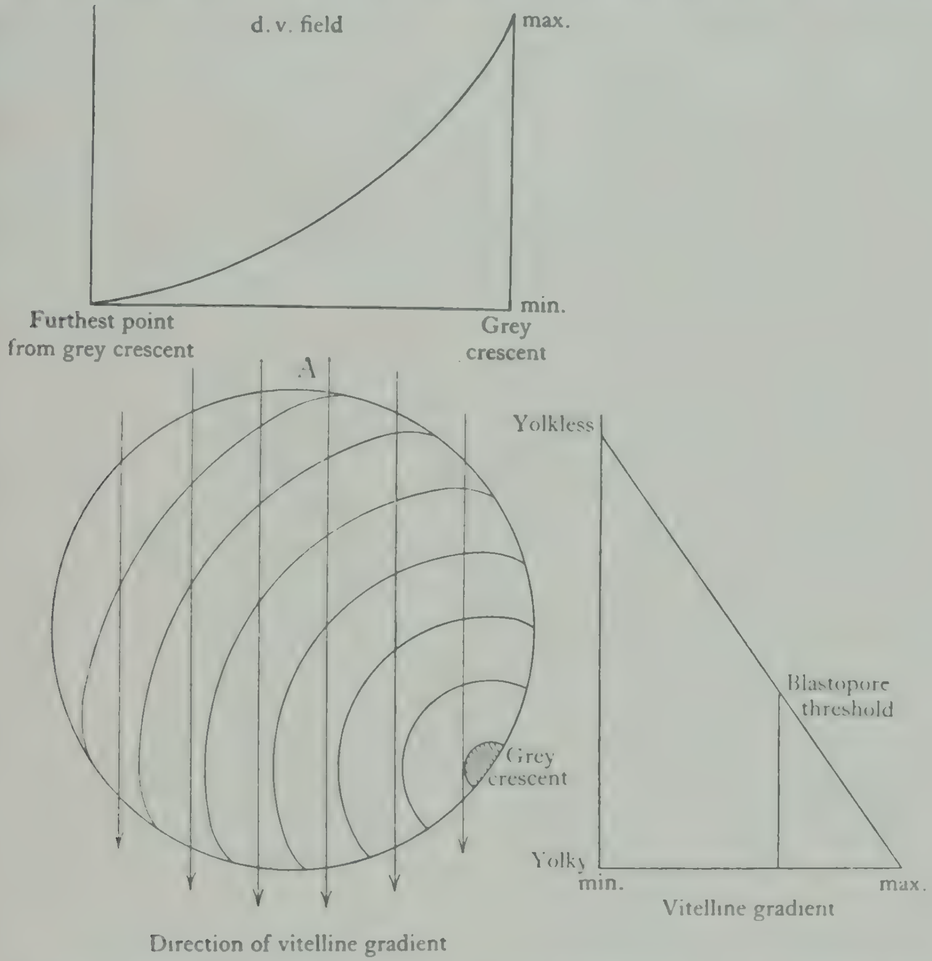
d. v. field
max.
min.
Furthest point from grey crescent
Grey crescent
A
Yolkless
Blastopore threshold
Grey crescent
Yolky
min.
max.
Vitelline gradient
Direction of vitelline gradient

Fig. 109. Diagram to illustrate the double gradient of the amphibian egg.

ne spermatozoon and the bilateral symmetry in the frog. As in the case of regulation question (see p. 99) the prince of theoreticians was beggared by difficulties of experiment. Investigations with more modern technique, how- r (T. C. Tung[1]; Pasteels[10]), have shown that there is no causal relation between point of entry and the position of the grey crescent. In some cases, on the ntrary, where a moderate statistical agreement may exist, it is likely that the n of bilateral symmetry determines to some extent the point of entry of the rmatozoon. A. Brachet[1], too, had shown that frog eggs parthenogenetically

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is always assumed that this is due to greater accumulation of yolk-platelets at the vegetal pole, recent work of Daniel[3] raises the suspicion that the continuous phase may be more responsible n the platelets.

stimulated by pricking with a needle showed bilateral symmetry entirely indepe of the point of pricking. Meanwhile the same independence of sperm entry had been conclusively demonstrated for the Urodeles by B. G. Smith; Vogt Bánki. It has even been shown by Ancel & Vintemberger[2] (who think th direction of the incident light has something to do with the determination plane of bilateral symmetry) that the plane may be altered after the spermat has penetrated far into the egg.

There has been much reluctance on the part of embryologists to give u spermatozoon as a determining factor in symmetry, probably because no causative factor could be visualised. But there is no reason for denyin maternal organism the power to construct a paracrystalline aggregate h quite complicated symmetry properties. The yolk gradient alone would b easy a task.*

Another factor which has sometimes been thought to be connected bilateral symmetry is the position of the first cleavage plane. The authors n above, however, and others, such as Ancel & Vintemberger[2], have shown there is no relation here; the grey crescent is usually unequally distrib between the first two blastomeres.

The key to the problem, as Dalcq[8] says,† is furnished by the work which followed the strange result obtained by O. Schultze in 1894, who discovered t frog's egg, when compressed and maintained upside down just after fertilisa and during the early cleavage stages, undergoes abnormal gastrulation, and often give rise to a twinned embryo. What happens is simple enough; under influence of gravity the yolk falls through the egg and comes to lie in the prev animal pole region. Later on the anomalous gastrulation takes the form Y-shaped or even double blastopores which invaginate to produce various t of monster, or else well-defined twins. When the experiments were repeate Penners & Schleip and Penners[3] on a larger scale thirty years later, it bec clear that there is *no* point on the egg's surface at which a blastopore may form. But it always forms at the junction between yolky and yolkless-pigme cells.

The meaning of these phenomena was not understood until Pasteels[11,] carried out a thorough reinvestigation of Schultze's experiment. It was t found that after rearrangement of the egg by drastic changes in the gradient, the blastopore always forms at the junction of yolky and pigme cells but oriented towards the original site of the grey crescent, tho this may have become invisible. Subsidiary blastopores may form late the side of the redistributed yolk-mass.‡ Blastopore formation therefore pends on two factors, (1) the contact between animal micromeres and yolk-c (2) the grey crescent. And as the effect of the grey crescent appears, tho

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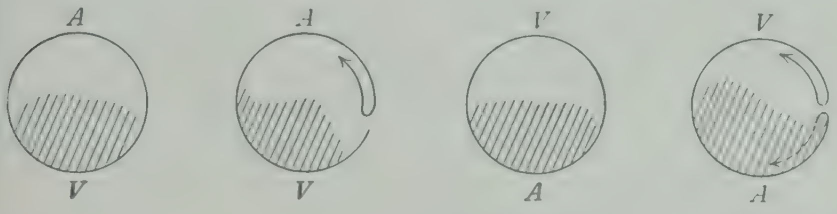
* In this connection it may be well to recall that in one group of animals at any rate, the ins we have known, since the work of Hallez in 1886, that the egg's axial orientation is exactl same as that of the maternal organism in the ovaries of which it was formed.

† In his *Form and Causality, etc.*, pp. 79 ff.

‡ They need not face the grey crescent, though one will, but may face the opposite direc The field determines their existence, not their orientation.

:r and slower, at remoter parts of the egg's surface, it is convenient to think · grey crescent as the centre of a field, rather than as the apex of a gradient. ese facts explained much that had previously been hard to understand. .ısion had primarily been caused by the simple-minded identification of grey :nt or corresponding region with the organisation centre. It was not ed that the organisation centre above the formed blastopore, with its liberated tor, may be a very different thing from the grey crescent with its masked .tor. There is indeed a pre-determination of the dorsal zone, but not a ormation of the organiser. Thus Fankhauser[2] had shown that ligatures of ıncleaved newt egg would produce the same results as those of Ruud emann in their classical constriction experiments on the gastrula (p. 103); ?asteels himself[2], by surgical destruction of the grey crescent region of the egg (following the pioneer work of Moszkowski in 1903), found inhibitions those produced by similar technique at the blastula stage by S. Suzuki. :e the too hasty conclusion that the active organisation centre itself, not merely lorsoventral gradient or field, was present in the earliest cleavage stages.



After inversion

Dotted line shows presumptive invagination

Fig. 110. Schultze's experiment in its extremest form (see text).

he importance of the junction between yolky and yolkless areas was likewise onstrated. Votquenne first showed that the micromeres of the frog's egg : VIII) form later all the presumptive mesoderm and hence the organisation e. Yet when Vintemberger[2] isolated them, no morphogenesis was produced. when he combined them[3] with a mass of yolk-cells from another embryo axial organs and structures later formed. Presence of yolk-cells was therefore 'n to be necessary for the formation of the organisation centre and the libera- of the evocator.

hultze's experiment was also performed on an oriental newt, *Hynobius 'natus*, by Motomura[2]. In this case the development is very slow, giving time ll the yolk to reach the former animal pole when the egg is inverted, before divisions prevent its redistribution. When the blastopore forms it forms ·ly in its normal place, but exactly inverted, and invagination goes on in ror image of normal invagination (see Fig. 110). This is the extreme case in ch normality is returned to after passage through a series of abnormal inter- liate types. In most amphibia, the yolk does not fall evenly through the egg, leaves streaks and irregular masses behind. Some will remain fixed to the ex at the former vegetal pole, and hence two blastopores will form in the grey cent field instead of one.

These facts have been made the basis of a general theory of early emb fields by Dalcq & Pasteels and by Dalcq[7] in his monograph. These authors it with much boldness to the development of all vertebrates and even of invertebrates. Whether or no their wider conclusions stand the test of tim certain criticisms, see Hörstadius[10]), it is sure that they are laying the found of comparative experimental, as opposed to morphological, embryology. view is briefly as follows. The animal-vegetal gradient (yolkless-yolky) they r as pervading the egg's substance; the dorso-ventral gradient they rega localised in the egg's cortex. But the dorso-ventral axis is thus a surface rather than a gradient, and Dalcq & Pasteels ingeniously derive many c phenomena of later development, including even the dynamically detern Vogtian areas, from the topography of the co-ordinate system so formed. picture the existence of some chemical reaction between the cortex and the (factors *C* and *V*), the product of which would be distributed in a regular w a series of thresholds to which the morphogenetic processes would be sens or competent. Thus they even describe the metamerism of the somites in t of the numerical values of the co-ordinates of their system. The blastopore form wherever the product of the reaction reaches a certain threshold. amount of the product is spoken of as the "morphogenetic potential".

The further details of this stimulating theory must be read in the orig Some of its detailed applications may appear questionable, but its fundame basis is not in doubt, namely that there is a yolk gradient, and that there grey-crescent field. The position of the blastopore, and hence of the libera of the evocator, undoubtedly depends on the interaction of this gradient this field (see Fig. 109).

One criticism interesting to us may be voiced. According to the Dalcq-Past theory, the grey-crescent field is located only in the cortex of the egg (*Form Causality*, pp. 83, 85, 146). At first sight the only evidence for this view se to be that the field is unaffected by maintained inversion and by centrifug And, as is well known (see p. 660), the echinoderm egg has symmetry and pola properties which are unaffected by the entire redistribution of the mova "ballast" and which have been thought to be referable to a paracrystal condition of the cytoplasm, not of the cortex only. A liquid crystal re-fo again with its molecules in characteristic orientation after the passage of a fore body. It may be, therefore, that the grey-crescent field runs also through substance of the egg as well as in its cortex. Against this, however, it may argued rather convincingly that in that case phenomena ought sometimes to met with in which an invagination would begin, not from the surface of the e but from some interior point at the junction of yolky and yolkless cells. But t never happens; the formation of the blastopore is essentially a cortex phenomen Furthermore, in Dalcq's view[7], once the field of "morphogenetic potentials" fully established, there is no doubt that it permeates the internal cytopla for different regions of it have different inclinations and different induc capacities.

Another criticism, due to Holtfreter,* is that if the presumptive organi cytoplasm had a specific gravity intermediate between that of the yolk and t of animal pole cytoplasm, it would always tend to take up a position betw them, and there would be no need to postulate a chemical reaction between yolk itself and other constituents.

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* In conversation. This is reminiscent of the Hippocratic writer's theory of development (p. 57).

e question may be asked whether the vitelline or animal-vegetal gradient
ny physico-chemical properties analogous to those which, as we saw in
on 2·2, are possessed by the severer localisations seen in mosaic eggs.
g to the pigmentation of most amphibian eggs, vital staining methods have
een available, but Dorfman[3] has applied electrometric technique. According
m, the vegetal pole is negative to the animal pole in the eggs of *Rana*
oraria and *arvalis*, changing sign at fertilisation, but as the eggs of
nta showed the opposite potential difference, and a correspondingly
site change of sign, our doubts are aroused. Later papers (Dorfman[4];
man & Grodsensky) give the following figures for polar material withdrawn
capillary tubes for measurement:

	Vegetal pole	Animal pole
*p*H	5·64	6·18
E_h in mv. (aerobic)	+262	+216

nal pole material would thus be more alkaline and have a more negative
ction potential (the E_h figures agree with an average for the whole egg given
. Brachet[6]). This is perhaps the kind of work which cannot be appraised
out a personal acquaintance with the technique used.

isorganisation of the embryo by centrifuging has been carried out by many
erimenters over many years, and even some chemical analysis of the redis-
uted materials has been done (*CE*, p. 347). We must, however, pass over the
ier work, and only mention results which bear on the present problems.
as the yolk, but not the grey-crescent field, is displaced by maintained
ersion, so centrifuging in the cleavage stages leads to double or even multiple
topores, and later to all kinds of monsters (Beams, King & Risley on *Rana*
ens; Ch'ou[2] on Chinese species; Konopacki[2] on *Rana fusca*; Motomura[2] on
o formosus). A case of double invagination without induction was observed
Beams, King & Risley, there being two notochords but no neural tube.
ilar duplications, particularly accessory tails, were seen on centrifuging
nced blastulae of *Hyla regilla* by Schechtman[5], and young gastrulae of *Rana*
by Pasquini & Reverberi, but in these later stages, after the organiser region
been formed, an abnormal contact between inducing mesoderm and com-
nt ectoderm will explain matters without the appearance of subsidiary
niser centres.

he best analysed centrifuge experiments are those of Pasteels[11,14]. The strongest
trifugation which the young morula can stand without cleaving abnormally
es three types of result: (1) inertia of the yolk-cells leading to spina bifida or
-gastrulation, (2) partial or total inhibition of invagination, leading to defi-
ncies in the head organiser, (3) invagination without induction. Only the
ond and third type need be mentioned. The invagination paralysis of the
ond type was shown by Pasteels not to be due to removal of glycogen from
dorsal lip area by the centrifuging, and its causation is obscure. The invagina-
without induction of the third type is of the greatest interest, and we shall
urn to it in the next section (cf. Fig. 116).

Dislocation of the normal relations of yolk gradient and grey crescent may be brought about by other means than the redistribution of the Witschi[1], in his studies on the development of over-ripe amphibian eggs, to we shall have occasion to refer again (p. 255), found that in some cases, mu blastopores were formed, giving rise to twins and various types of monsters. the yolk had not been redistributed, this effect would appear to be one direct the grey crescent field, possibly a decay of the original dorso-ventral organis of the egg; a reversion to randomness of molecules previously oriented in s

2·37. Anomalies of organiser function

2·371. Organiser inhibition

In the foregoing section the normal process, where liberation of the evo accompanies blastopore formation, has alone been considered. But if the fo could be inhibited without the latter, invagination would take place bu induction of neural structures in the overlying ectoderm. Such an effec been several times described.*

The earlier localised irradiations of amphibian embryos with ultra-violet li (W. M. Baldwin; Dürken[3]) produced little of interest,‡ but in later work Dür succeeded in obtaining a few clear cases of invagination without any sig induction after localised treatment of the dorsal lip of the blastopore with ul violet light. In his discussion he envisaged only the actual destruction of evocator substance and thought it strange that a substance so stable to m chemical treatments should be sensitive to ultra-violet rays. In view of classical formation of calciferol (vitamin D) by irradiation of ergosterol, thi perhaps not so remarkable. But even more probably the radiation may supposed to inhibit the liberation (activation) processes. The effect of ultra-vi rays on carbohydrate catabolism appears to be complex, for while Stiven fou increased lactic acid production in muscle extracts, a destructive effect up amylase was observed by Kumanomido and by Nadson & Stern. Perhaps t may be resolved by findings such as that of Hutchinson & Ashton that shor wave-lengths of ultra-violet destroy amylase, while longer wave-lengths celerate its action. A formal analogy to inhibition of evocator liberation mi be derived from the work of v. d. Laan on auxin, the formation of which fr its precursor is inhibited by ethylene.

Reith[4] subsequently tested the inductive capacity of dorsal lip pieces whi had been irradiated with ultra-violet *in situ*, and found, probably because range between complete inhibition of the liberating processes and necrosis ı is very narrow, that in all his cases the inducing power was present.* viour in the host, however, showed certain peculiarities which will be men- ·d again later in connection with regional differentiation (p. 277). Similar . is being continued by Brandes.

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* Of course in every *Bauchstück* (see p. 103) mesoderm gets inside but as it comes from ventral lip it does not induce.

† Other references to the effects of various forms of radiation on embryos will be found later the present book, e.g. in connection with inherited abnormalities (pp. 368 ff.). The mere establis ment of variations in susceptibility during development received some treatment in *CE* (Section 1 and will not be taken up again here because its interpretation remains quite enigmatic.

‡ Except in regard to the origin of *spina bifida*, which, as Baronovsky & Schechtman ha now shown, can be produced at will by localised irradiation of the lateral blastopore lips anurans.

the preceding section it was mentioned that Pasteels[11], by carefully dosed rifuging during the cleavage stages, also obtained invagination without ction, no trace of notochord or neural plate being visible. The only indication e dorsal surface of these embryos was the excentric position of the endodermal Now in the work of Brachet[18], which was proceeding at the same time, it was ıd that the distribution of fixed –SH groups was quite different in the centri- ·d eggs from the normal. The protein carrying them passed, unlike the yolk- elets and the pigment, to the animal pole, together with the fat and aqueous ·plasm. Hence in the dorsal lip of embryos invaginating without induction, e of the protein was to be found. This seems to be almost complete proof the protein plays a part in evocator liberation, and might indeed itself be protein of the masking complex.

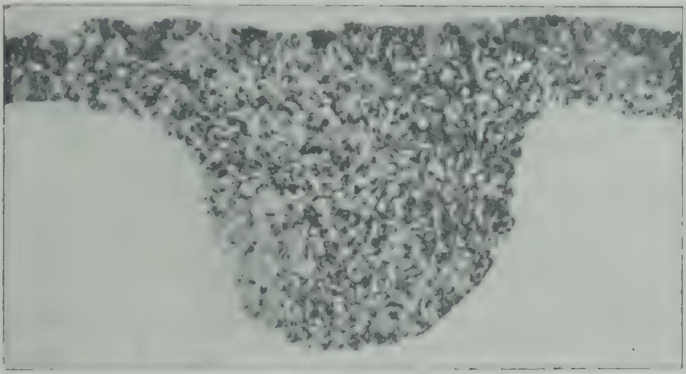


Fig. 111. Cross-section through an anidian blastoderm (chick).

The primary organiser may also be suppressed in the sauropsid egg (see p. 330). ıas long been known that in the sauropsida, there are occasionally found stoderms where an active proliferation of cells goes on but no trace of axiation ·ears. Primitive streak and neural groove never form. These blastoderms are ed *Anidians*, and have recently been described anew by Grodziński[3]. It is remely likely that in these cases there has been a failure either of the formation, more probably the liberation, of the primary evocator. The natural cause of ·s is unknown, but X-radiation will produce them (Ancel & Vintemberger[1]), ıl Edwards long ago was able to obtain anidians regularly by incubating hen's ȝs at subnormal temperatures. This is the usual way of obtaining them. The ·rk of Tur[2], to be referred to again in connection with cancer (p. 258), on the traordinary ectodermal thickenings of some early lizard and bird blastoderms, also interesting in this connection, for these never develop an embryonic axis 'ig. 111). Anidians may develop an area vasculosa.

In the *Annual Rev. Physiol.* 1938 I suggested that this result implied that Dürken's effect had en on the competence of the presumptive neural region rather than on the evocator. But as ; irradiations were localised to the dorsal lip of the young gastrula, i.e. to presumptive mesoderm, is explanation can hardly hold good.

Perhaps the most striking instances of the effect of a definite external influ in suppressing organiser action have come from the line of work pursue F. E. Lehmann. Lehmann discovered a number of specific inhibitions of mor genesis produced by artificial agents which leave all the surrounding ti unaffected. The theoretical basis for such work lay in the conviction that n the tissues or organs of the body pass through their points of maximum gr rate or the most difficult phases of their development at one and the same ti There exist "critical points" for each structure (Stockard; and *CE*, p. 14 Thus Lehmann[5] found that trichlorbutyl alcohol (the same narcotic which, as saw (p. 165), has no influence on the primary evocator) can abolish specific the second-grade evocation of the lens by the eye-cup in amphibia (see pp. 291 ff.). Fig. 112 shows three of the results he obtained; on the left an embryo maintained in a strong concentration of the narcotic, in the middle an embryo maintained in a weaker concentration of it; and on the right a control embryo. It will be seen that the lens is entirely suppressed on the left, abnormally small in the middle, and normal on the right. Between the size of the lens induced and the concentration of the narcotic there was a far-reaching parallelism. Fig. 113 shows the relation between lens size and concentration of narcotic when the period of application of the narcotic corresponded to the neurulation period. At other periods the effects were less marked and less regularly dependent on concentration. After the appearance of the lens-forming ect dermal thickening the process of lens-formation could no longer be inhibite Lehmann[9] went on to study the effect of another variable, temperature. H found that the inhibitory effect was more pronounced the lower the temperatu (between 10 and 22°).

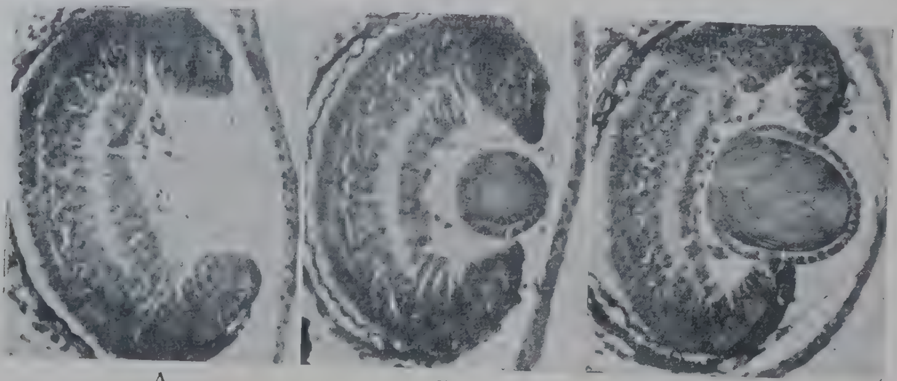


Fig. 112. Suppression of lens-induction by trichlorbutyl alcohol.
A. 1 in 900. B. 1 in 1200. C. Normal.

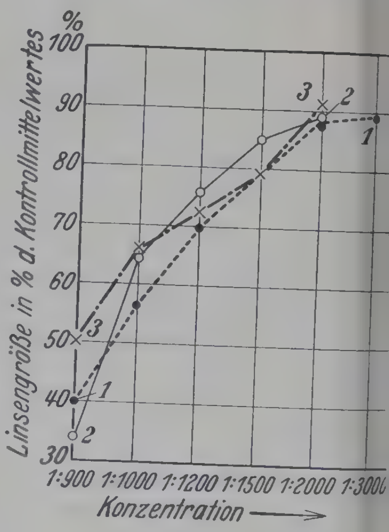


Fig. 113. Relation between c centration of narcotic and ext of lens-suppression.

As regards the mechanism of this inhibition no definite decision is as y possible, but Lehmann[8] regarded it as a diminution of ectodermal competen ner than an action on the inducing substance or its precursor. He recalled t according to Manuilova & Kislov the frequency of successful lens inductions eye-cups in belly epidermis is not so high as in the normal lens area, suggesting ower level of competence in the unusual situation.

These facts raise the question of the naturally-occurring blindness of cave-elling animals. There can be little doubt that it involves a series of effects due nterference with the inductor chain brain→eye-cup→lens→cornea (see p. 290) various different points. Thus Schlampp found that in *Proteus anguineus* a ck wad of connective-tissue mesoderm intervenes between the eye-cup and the dermis, inhibiting cornea induction. In other forms, such as the blind cave-n *Anoptichthys jordani*, there is a spontaneous failure of lens-induction resser & Breder). From the work of a considerable number of investigators g. Eigenmann; Eigenmann & Denny, etc.) the blind forms can be seen to esent a graded series ranging from (1) normal development followed by ondary degeneration of the retina, through (2) considerable size-reduction of eye accompanied by overgrowth of the eyelids,* and (3) partial or drastic lure of induction of eye-cup, lens, or cornea, to (4) complete absence of the s or cornea and failure of eye-cup differentiation. Such a series of fishes uld be *Lucifuga-Chologaster-Amblyopsis-Troglichthys-Typhlichthys-Anoptichys*. A similar series among blind amphibia would run: *Spelerpes-Typhlotriton-phlomolge*, and lizards such as *Amphisbaena* and *Rhineura* lack lens and cornea. terference with the visual inductor chain occurs also in abnormalities due to hal or semi-lethal genes, to which we shall return in Section 2·65, and in clear abnormalities such as androgenesis (Porter; see Section 2·64). It must be nembered that among the inverte-ates also there are many blind cave-ms, the literature on which may be proached by way of Wolsky's interest- paper[5] on the blind gammarid *hargus*.

Results of interest in this connection those reported by Solberg[2], who diated eggs of the teleostean fish *zias* with X-rays. A suitable dose l almost completely suppress the velopment of the eye-cup, but parently not before it has exerted ficient lens-inducing stimulus on the ectoderm. Solitary lenses thus appear, minding us of those found in Holtfreter's experiments (see p. 170): Fig. 114. he suppression of the eye-cup is associated with a spherulation of the cells, and is is also the case when the neural tube is caused to degenerate by X-radiation. itable doses would also suppress the appearance of gills and mouth, but not e ear-vesicle.

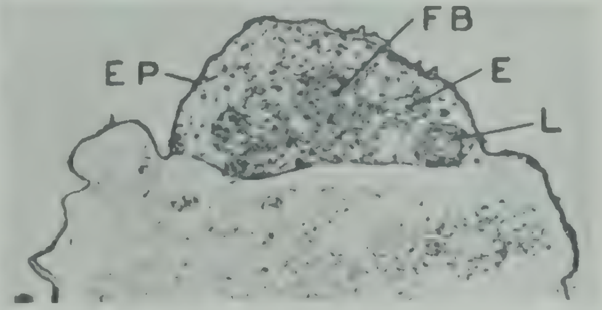
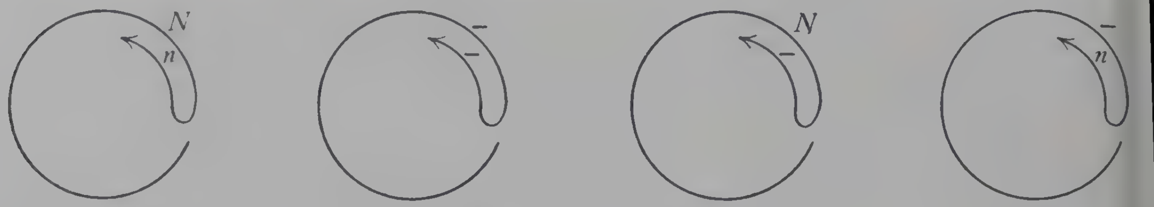


Fig. 114. Suppression of eye-cup and brain development by irradiation of teleostean embryo with X-rays, without suppression of lens-induction. *EP*, Epidermis; *FB*, forebrain; *E*, eye debris; *L*, lens.

In preliminary experiments, Twitty[7] has been able to produce this overgrowth of the eyelids immobilising the eye-ball or substituting one of a smaller species.

Another remarkable finding of Lehmann's[6] was that by the action of suit concentrations of lithium* and potassium chloride, embryos could be prod completely lacking notochords. Fig. 115 A (opp. p. 234) shows one of these. Al is the normal cross-section, showing the somites on each side of the notoc and the neural tube above it; below is one of the notochordless embryos. Fig. 1 illustrates a later stage of development. The surrounding morphological histological differentiation looks remarkably normal. Here there has evide been no interference with the primary evocator, but the differentiation of invaginated mesoderm has been profoundly altered. In the ultra-violet irra tion experiments the formation of the notochord was eliminated as well as primary induction of the neural axis.

The absence of notochord was found by Lehmann[13], however, to lead in m cases to quite abnormally formed neural tubes (see later, under *Nachbarsch* pp. 284). Frequently the spinal ganglia developed normally. More surp ingly, Lehmann & Ris observed a much delayed but fairly normal developm of cartilaginous axial skeleton in the regions where the notochord was absen



Normal

n = notochord
N = neural tissue

Durken's case
[irradiation by UV]
Pasteels
[after centrifuging]

Lehmann's case
[lithium]

Case of
Beams, King & Risle
[after centrifuging]

Fig. 116. Cases of intereference with primary organiser function in amphibia.

Full investigation of the missing notochord region by Lehmann[13] with vital dy showed that the presumptive notochord material had been converted wholly in somite material.†

The converse of Lehmann's notochord suppression was obtained by Ranzi Tamini using sodium thiocyanate (an "animalising" agent in the echinoder see p. 486). The abnormally large notochords were explained by these auth as due to inhibition of the protein metabolism of regions surrounding the dor lip, while leaving carbohydrate metabolism in the lip itself unimpaired.

Lehmann has interpreted the general significance of his findings in seve interesting reviews[8,10,11]. The logical relationship of the cases of interference w primary organiser function in amphibia is shown in Fig. 116.

* The strange morphological effects of Li' will often be referred to in succeeding sections (pp. 277, 283 and 486). The ion also induces manifold abnormalities of shape in bacteria (End lein; Kühn & Sternberg; P. Hadley).

† The legitimacy of speaking of this process as "mesodermisation", as Lehmann[13] does, is quite clear, since both notochord and somites are generally regarded as mesodermal. He anxious to approximate the effects of lithium on amphibian to those on echinoderm developm (see pp. 477 ff.), but it is perhaps as yet too early to attempt this until we know more about metabolism of both these groups in the gastrulation period. Cohen, if I understand him arig thinks such missing notochords may be "endodermised" and lost in the gut roof.

Anatomical and genetical literature is indeed full of cases in which the stimulus the development of a certain structure has failed. For example, the paper of nkmeijer describes the congenital complete absence of the tibia in man. Not y may the inductive stimulus of eye-cup on ectoderm fail; there may be a ure of those as yet unknown factors in regional differentiation which bring ut the formation of the eye-cups themselves. Such failures were discussed v. Baer[6] already in 1862, who saw their significance for cave-blindness and erited anomalies.

Among external causes of this, vitamin A may be mentioned. Vitamin A is a ochrome allied to carotin, astacin, and the retinene of the eye; and its structure hat of a long carbon chain, containing four unsaturated linkages, and ending in exatomic ring with one double bond (see p. 249). Information is now beginning come through that this vitamin may be connected in some way or other with formation of organs in embryonic development. Thus it seems that in the sence of sufficient vitamin A from the diet of some mammals during gestation, re may be a complete failure to form the eye-cup, and hence no induction lens from the adjacent ectoderm. In 1932 at the Texas State Agricultural periment Station a Duroc-Jersey gilt ·receiving a vitamin-A deficient ration rowed eleven pigs, all of which were completely devoid of eye-balls. Hale, o reported the case, calculated that the chance of such a result being due to eritance was exceedingly small, and he was subsequently able to repeat the ult with other gilts, and sows, by placing them on a ration containing insuffint amounts of the vitamin. Fig. 117 shows one of the pigs born with complete sence of the eye-cup.* Such pigs as had eye-cups suffered from defects of ion, including total blindness. In view of the fact that vitamin A is closely ated chemically to the lipochrome constituent of visual purple, the experiments ve additional interest.†

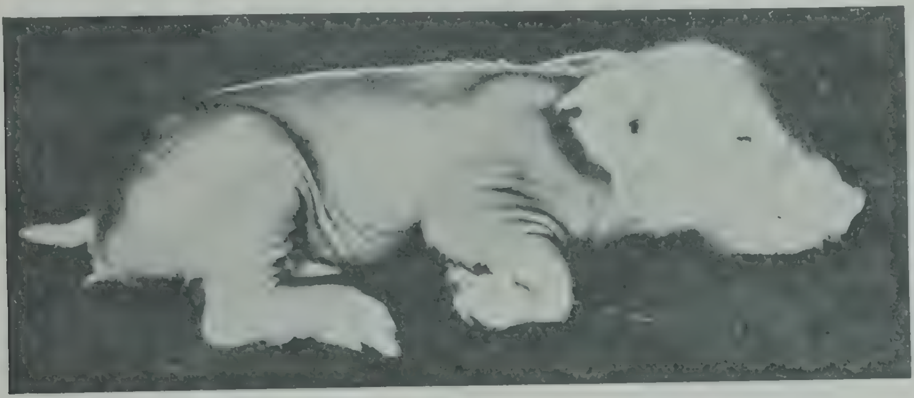


Fig. 117. Suppression of eye-cup formation in the pig in vitamin A deficiency.

Lack of vitamin A has also been shown to be responsible for a disturbance the mechanism of normal limb-bud formation. In 1921 Zilva, Golding,

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"Eyeless" rabbits are sometimes ascribed to Guyer & Smith, but their work only involved tain apparently inherited defects such as opacity of the lens. In *Drosophila*, of course, there is vell-known recessive gene for "eyeless" (see p. 376).

Wolbach, in an interesting lecture, has emphasised the relation between vitamin effects and bryological problems.

Drummond & Coward found that on an A-deficient diet some of their sows p duced little pigs in which the limbs were completely missing. Fig. 118 sh how the hind-limbs are represented by nothing but minute stringy appendag

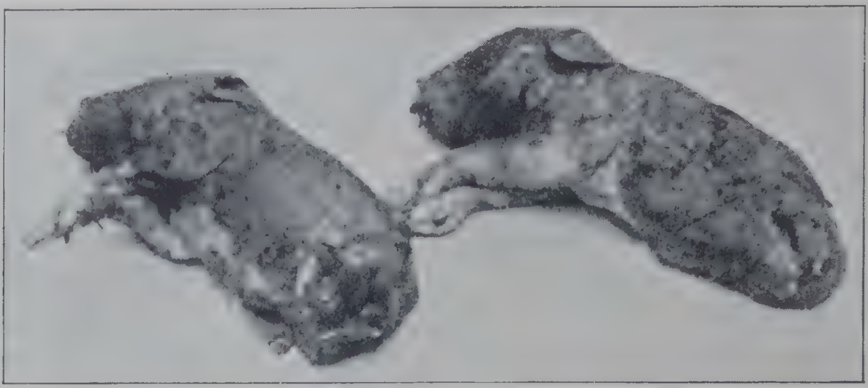

Fig. 118. Suppression of limb-bud development in the pig in vitamin A deficiency.

2·375. Organiser excess; Twinning

If the grey crescent field of an amphibian embryo, or the individuation fie of a chick, for example, were to split into two, the primary evocator would b liberated in active form at two places instead of one, and a twin would resul Such phenomena must lie at the basis of many kinds of twinning and poly embryony. The literature on this subject is a large one; v. Baer[4] worked o it over a century ago, and in recent times we have the monographs of Newmar (though written before 1924) and the review of Patterson.

In the ordinary way, one egg gives rise to one embryo. But in polyembryon one egg may give rise to a number of embryos, even up to two thousand, if w take the case of some parasitic insects. This must ultimately be related to th fact that, as we have already seen, the destinies of the parts of the embryo a not fixed at the beginning, but only become so as development proceeds, owin to the ordered action of a series of determining agents, or organisers. Whi identical twins or quadruplets are a relatively rare occurrence in most specie polyembryony of a much higher order has established itself as a specific charact in a few. This may involve either the retention of pluripotence through man cleavages, followed by separation of blastomeres, or the scission of the individua tion field at a later stage followed by the appearance of many organisation centr instead of one. It seems that the exact time of splitting varies very greatly i different organisms. Thus in the earthworms, such as *Lumbricus trapezoide* there takes place a curious kind of double gastrulation so that the two gastrul have their blastopores facing, and presently break apart (Kleinenberg[1]). In th parasitic hymenoptera (*Platygaster*, *Litomastix*, etc.), the processes are very od and clearly highly specialised, but may roughly be described as the separatio of many early blastomeres, followed by isolated differentiation (Leiby). The ca of the Texan nine-banded armadillo (*Dasypus novemcinctus*), which normal produces four, and occasionally five, identical-twin embryos from a single egg, the closest to our present discussion, for here the blastocyst, which is similar i y respect to that of other mammals, regularly develops four embryonic axes ead of one. The deduction that a highly controlled liberation of the primary ator at four separate points instead of one takes place is too plausible to be bted.* In Fig. 119 is shown a diagram of the armadillo blastocyst illustrating description of Newman & Patterson.

n human monozygotic (identical) twins, a common chorion and sometimes n a common amnion is found. This is regarded by Arey as proof that the nning process occurred subsequently to the differentiation of the ovum into inner cell-mass and an outer trophectoderm, as it would do if there was a uplication of the primary organiser. The only flaw in this argument is that, ording to Greulich, chorionic walls may fuse over the area where they meet. e presence of only one chorion at birth, therefore, does not absolutely disprove view that a separation of blastomeres occurred.

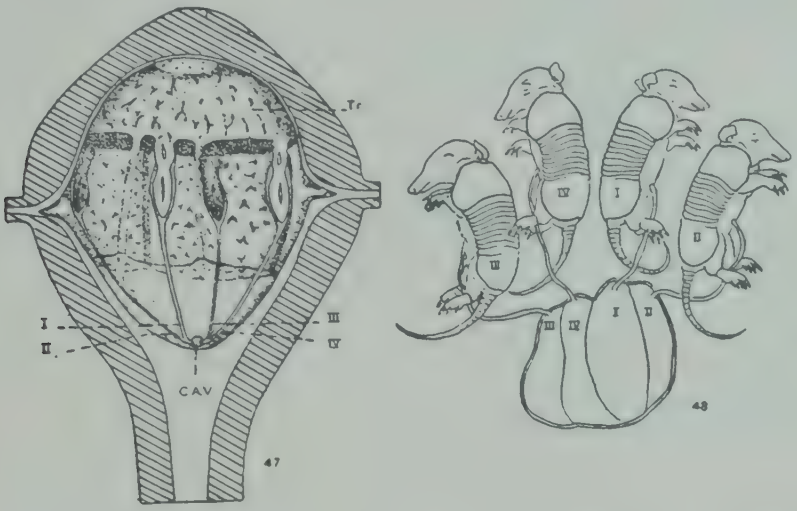

Fig. 119. Normal formation of identical quadruplets in the armadillo.

What happens normally in the armadillo happens occasionally in other mammals in birds. Assheton[1] figures two entirely separate germinal areas in a blastomic vesicle of the sheep, and the later results of lesser degrees of separation be seen in the review of Keller & Niedoba. O'Donoghue photographed a k blastoderm in which two quite separate embryonic areas had developed, g head to head, while Tannreuther found two distinct embryos only united he extreme posterior end of the primitive streak (Fig. 120). The parallel ween such a picture and that of a blastoderm in which a secondary embryo has n *induced* artificially by the transplantation of an organiser is striking (see . 121, opp. p. 234, from Waddington & Schmidt). The latest experimental study twinning in bird blastoderms by injury is that of Twiesselmann, who gives the evious literature.†

This would be called in the old terminology "fission", not "blastotomy" or "budding". wman[1] spoke of the "isolation of four growing-points". That this comes about through temary developmental arrest in accordance with theories of earlier American authors is unlikely, Hamlett[1] has shown that the quiescent period happens at the wrong time. The general views ressed in this section have since been advocated also by Dalcq[13].

The interesting reviews of Hildebrand and of Strohl[2] give details of natural twinning in turtles snakes; Tung & Tung[2] describe it in anurans.

It is interesting in connection with evidence to be given later regarding gene
factors, that this division of the individuation field into two or more fields
be under the control of inheritable factors, since according to Davenport[1],
production of human identical twins appears with great frequency in cer
families, and may therefore be connected with a specific gene or gene-gr
Against this there is the conclusion of Greulich's recent review, that only dizyg
(fraternal) twinning in man is hereditary. But for birds it has been shown
Byerly & Olsen[2] that the incidence of twinning varies according to the br
a fact which will be difficult to explain on other than genetic lines.

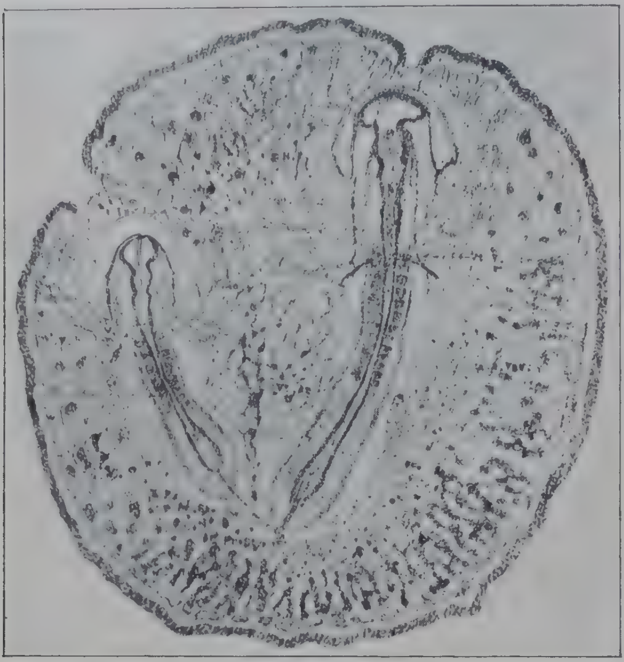

Fig. 120.

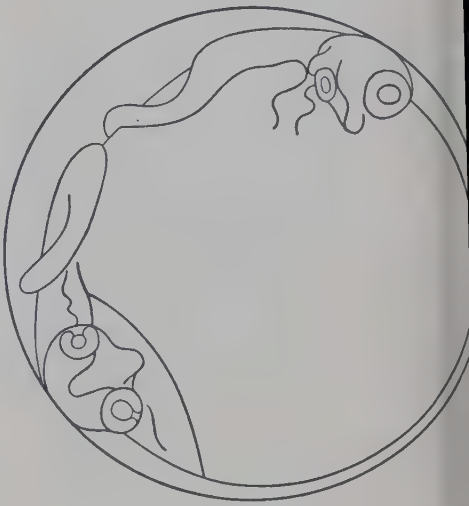

Fig. 122.

Fig. 120. Naturally occurring complete twinning in the chick, probably due to separate en
derm invagination.

Fig. 122. Twinning in the minnow produced by ultra-violet irradiation.

The study of developmental abnormalities also indicates that the extreme t
of twinning may be brought about by various influences. Without pursuing
large literature on this subject, we may refer to the paper of Harman on
appearance of double notochords running parallel in chick embryos after incu
tion at abnormally high temperatures in the early stages. Fish embryos seem
be particularly prone to scission of the individuation field and production of t
organiser centres instead of one. Stockard, in his well-known studies on crit
points in development (see *CE*, p. 1409) produced all varieties of twins in tro
minnows, etc. by the action of magnesium chloride, insufficient oxygenation a
other means.* Ultra-violet irradiation, too, will produce twins complet
separated, and hence not to be ascribed to interference with gastrulation mo
ments (Hinrichs and Hinrichs & Genther on *Fundulus heteroclitus*); see Fig. 1
Finally, Enzmann & Haskins[3] find that neutron bombardment produces all ki
of reduplications of organs in *Drosophila*, quite unlike the effects of X-rays.

* Cf. the new analysis of fish twins and polyembryos by Lynn.

·376. Organiser excess and anomalous competence; Teratomata

e now come to the Teratomata. We must first of all make a distinction which
ometimes overlooked in the literature. The word τέρατα in Greek means
nsters, in the sense of signs and wonders, like *prodigium* or *portentum* in Latin.
s a commonplace of embryology that terata may be produced by every
ceivable kind of deviation from normal development, and their causation is
ainly very unspecific. On such deviations the monograph of Dareste, though
y years old, is still classical. But terata are not teratomata. A teratoma may
defined as a more or less malignant assembly of tissues, well differentiated
ologically, but showing varying degrees of morphological differentiation, and
bedded in the body of an otherwise normal fully developed organism. The
lly typical character of such an assembly is its approximation to chaos; as if
tissues and morphological forms had arisen from the interplay of almost
irely unregulated forces—resulting in the state described by Schiller:

> "wo rohe Kräfte sinnlos walten,
> da kann sich kein Gebild gestalten".
>
> (*Lied von der Glocke*)

he literature on the subject of teratomata is extremely large. The writings
athologists on teratomata, moreover, seem to have been marked by an unusual
ree of unscientific speculation, inaccurate description and logical mistakes,
may be seen from the long and remarkable series of critical reviews by
holson[1,2]. A great deal of this, however, may perhaps be put down to the fact
t for the last fifty years, at least, pathologists have approached the study of
atomata wearing, if we may say so, the spectacles of an unjustifiable hypothesis
ut the nature of these tumours. This hypothesis is expressed in the words of
nd-Sutton[2], who, defining teratomata, says: "A teratoma is an irregular
glomerate mass containing the tissues and fragments of viscera belonging to
uppressed foetus attached to an otherwise normal individual." As the result
this basic misconception, chaotic and truly innominate structures have been
n the names of nipples, limbs, vulvae, jaws, and all manner of dignities to
ch they really have no claim.* And this is all the more unfortunate as in
t cases the brief anatomical descriptions have not been accompanied with
thorough histological analysis which the study of serial sections alone can
.

efore going further, it would be as well to make the discussion more concrete
giving a few examples of the kind of phenomenon we are considering. First
ll, as is generally known, the morphological forms attained by teratomata may
very complete. Fig. 123 shows an ovarian dermoid teratoma. According to
nd-Sutton's description[3], it was removed from a single woman twenty-six
rs of age. It was the size of a coco-nut and contained a mixture of grease and
r. At the lower pole of the tumour there was a patch of reddish epidermis
ered with reddish hair, and out of which appeared two teeth, an incisor and
icuspid, fully erupted. Underneath this skin was a mass of nerve tissue. The
ge size of such cysts is supposed to be due to the pressure of the sweat and

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he homologies of Wilms, who wrote in 1895, resemble nothing so much as the unfortunate
s of William Croone (1672), whose identification of a torn piece of vitelline membrane
the preformed chick embryo has recently been unkindly made public again by F. J. Cole.

grease secreted over a long period of time,* and the growth of the hair. presence of well-formed teeth in teratomata is not uncommon; Fig. 124 show X-ray photograph of an ovarian one, described by Sprawson.

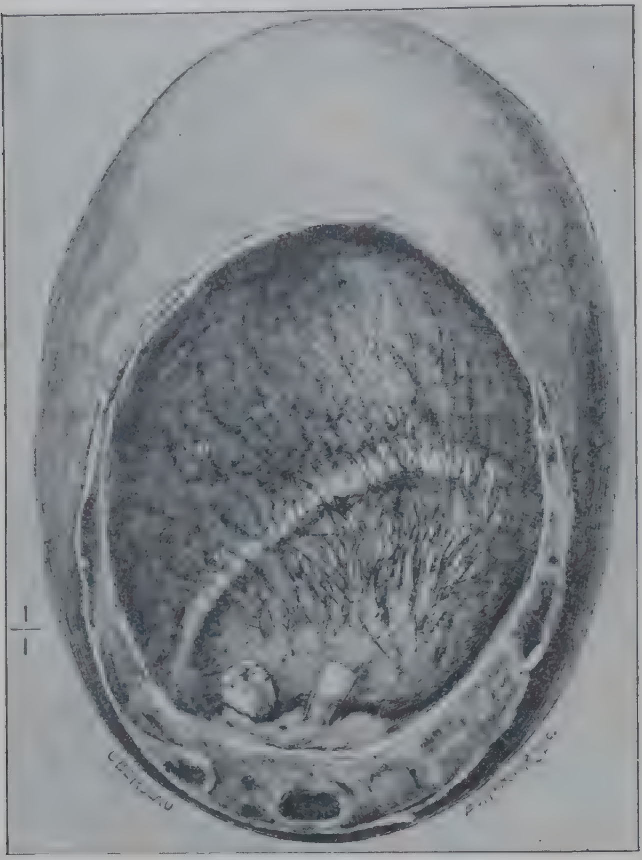

Fig. 123. Ovarian dermoid teratoma (human).

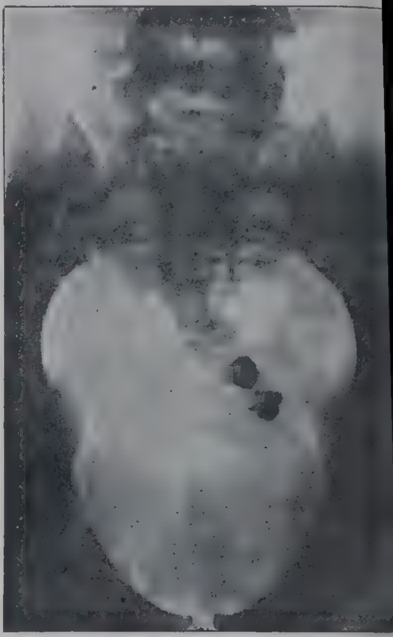

Fig. 124. Well-formed teeth in ovarian teratoma (X-ray photogra

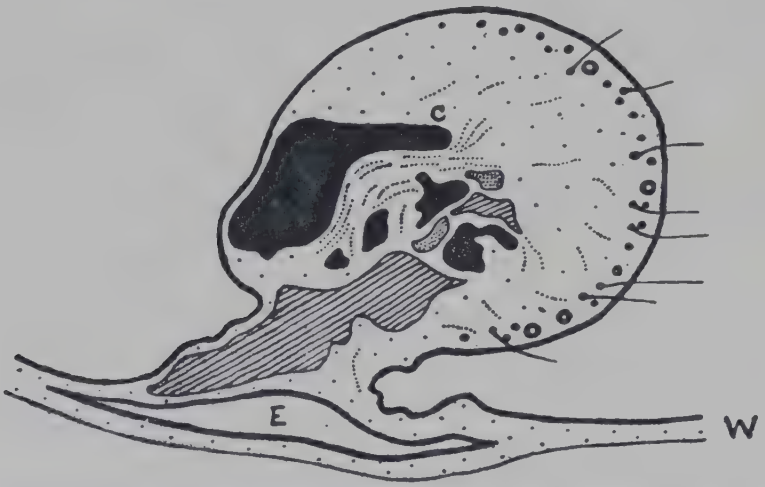

Fig. 125. Tissues in an eminence within an ovarian dermoid teratoma. Black = bone; shade neural tissue; stippled = ganglia; dotted lines = connective tissue; *C*, cap of cartilage; *E*, c in *W*, cyst-wall.

The same chaotic mixture of tissues occurs in a case described by Willis[2], w gives the instructive cross-section shown in Fig. 125. This is a section of inence which projected into an ovarian dermoid cyst about the same size as t just described. The tumour originated from a single woman aged thirty-eight. an be seen that the eminence contained bone, central nervous tissue, ganglia, ves, and connective tissue. At one point the bone had a cap of cartilage. other of Willis' cases had masses of cerebellar tissue with beautifully differ- iated Purkinje cells. But in these pictures, as in all teratomata, the general racter is the same, namely a complete absence of organisation of all these tissues o a whole, or we might say, an absence of individuation.

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* The fatty material in ovarian dermoid cysts may be of a very odd composition; Behmel fi large quantities of cholesterol esters in it but no vitamin A or sex-hormones, while Dim isolated 20 % of squalene, or some very similar hydrocarbon, from it.

The following case provides an opportunity for the transition to experimental bryology. Barnard described a multiple teratoma of the peritoneum in a man of forty, probably originating from an ovarian dermoid cyst. The owths, irregular, nodular, and partly cystic, occupied most of the internal face of the peritoneum, and when sectioned revealed the usual chaotic forma- n of tissues. There were undifferentiated cells, spindle cells arranged in groups d bands, islands of cartilage or trabeculae of bone, squamous and columnar ucus-secreting epithelium, and well-developed brain tissue. In one of the asses on the omentum a perfectly formed tooth was found. In Fig. 126 is shown typical collocation of tissues; in one and the same section can be seen brain sue, nerve ganglion, cartilage, and squamous epithelium.

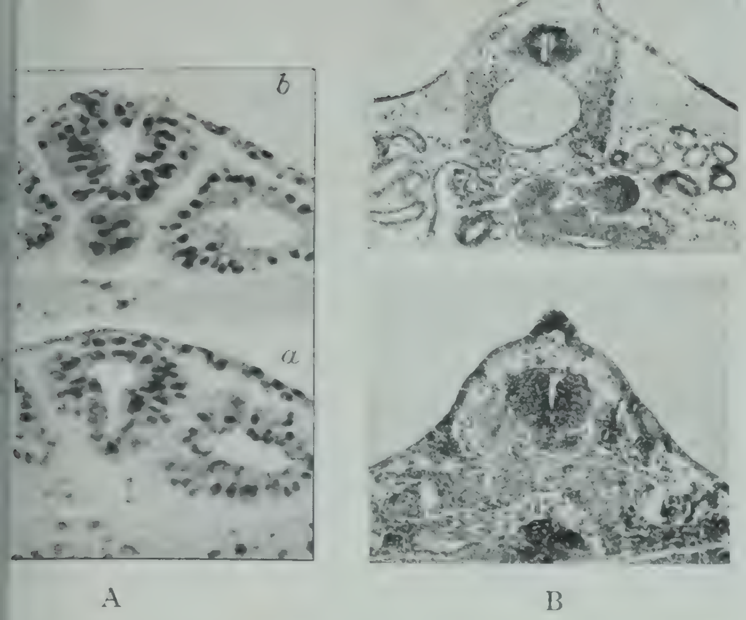
Such a picture at once calls to mind the kind of chaotic distribution of tissues hich is seen when for some reason the individuation field in a young embryo thrown out of gear. As we have seen, the normal induction of the primary axis an embryo by a living piece of organisation centre involves both evocation, e stimulus of a specific hormone-like chemical substance, and individuation, e regional differentiation of the axis so called into being. In all probability dead piece of organisation centre carries the evocator but not the individuation ld (see below, p. 277). It might therefore be expected that the implanta- n of a dead organiser would call forth the appearance of more chaotically anged structures than that of a living one, always provided that the individuation d of the host was not sufficiently strong to control and order the newly pearing differentiations. Let us therefore look at Fig. 127, which is taken from ltfreter's work[12] on the distribution of the evocator in the animal kingdom e p. 172). Embryo extract was prepared from chick embryos as is usual in sue culture work, coagulated by heat, and then implanted into the blastocoele vity of a newt embryo. Here in due course two neural tubes were induced, ree separate lots of notochord tissue, several somites, and at least two ear- sicles. Or consider Fig. 128, in which is shown the disorderly but impressive ray of no less than five ear-vesicles induced in the ventral ectoderm of a newt nbryo by the implantation of a small piece of heat-coagulated mouse liver. urrounded by these lies the great irregular induced brain (Holtfreter[7]).

It will surely be admitted that these parallels are too striking to be illusory. hey lead us directly to the basic assumption that what lies behind all the strange henomena which we see in the teratomata is the failure of the individuation ld, at some point early in development, to control the action of evocating bstances.

It is interesting that there has long been an intuition that teratomata w
not be explained until our knowledge of experimental embryology was m
further advanced. Thus Benda in 1895 attempted something in this direct
but at that time so many concepts subsequently found to be misleading (s
as post-generation and universal mosaic development) were in the field,
little could be done. The notion of "formative stimuli" had, it is true, b
advanced, but nothing, or nearly nothing, was known about them (see pp. 103
It seems that the credit of first realising the application of our knowledg
organiser phenomena to teratomata should rest with Budde, who only two y
after the critical experiment of Spemann & H. Mangold wrote a paper al
these lines in describing a case of teratoma. What he called an "abgespren
Organisator" might, he thought, have been at work. Later on Nichols
Langdon-Brown, and doubtless others, have realised that organiser phenom
provide the only basis for a modern theory of the origin of teratomata. "W
I miss", wrote Nicholson, "in the established teratoma is the co-ordinat
action of the whole upon its parts; in more scientific language, evidence for
action at any stage of development of the dominant organiser for a body."

If, then, we can only explain the occurrence of teratomata as the result
un-coordinated organiser action, it is none the less necessary to point out t
besides the stimulus there is also the reacting tissue. In addition to the evocat
we must also take the competence into account. Obviously we have to consid
two fundamentally important factors: firstly, the liberation of evocators of fi
second, third, or lower grades at times and places where they are not norma
liberated; secondly, the persistence of competence to respond to them beyo
the time at which it is normally lost. We know that the primary evocator is prese
in most adult tissues of animals in the free state. It is therefore likely that t
second of these factors may be more important for the formation of teratoma
than the first. But it is evident that from them both, by all sorts of combinatio
and permutations, everything that we know of under the name of the teratoma
may be produced.

There remain for consideration the reasons for which in some cases a terato
manifests malignancy. As Willis[1] and Nicholson[2] point out, the majority of te
tomata are benign rather than malignant. Ovarian ones are generally beni
while those of the testis are almost always frankly malignant, "seldom faili
to yield metastases and to kill their hosts". It is noted that when a beni
ovarian tumour becomes malignant, the change affects only one component, su
as the epidermis, so that a squamous-celled carcinoma quite similar to a
epidermal carcinoma is produced. In the testis, on the other hand, the malignan
of the teratoma is a property of the whole growth, so that metastases frequen
exhibit two or more components of the primary growth. Occasionally, as appea
from one of Willis' cases, one component of an otherwise relatively quiesce
teratoma of the testis "assumes disproportionate powers of proliferation" a
invades the remainder as a malignant neoplasm. There can be no doubt that t
terms "benign" and "malignant" are but relative, and that there may be grad
of malignancy depending ultimately on the inherent growth energy of the vario
components; thus in another of Willis' cases, the neuro-epithelial tissue appear
to be growing more rapidly than the glandular portion, while that in turn w
outstripping the cartilaginous material.



115. Suppression of notochord formation by lithium.
A. Early stage. B. Late stage.

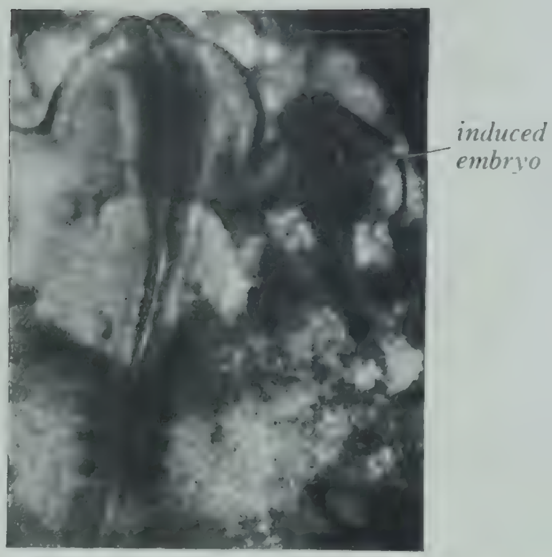
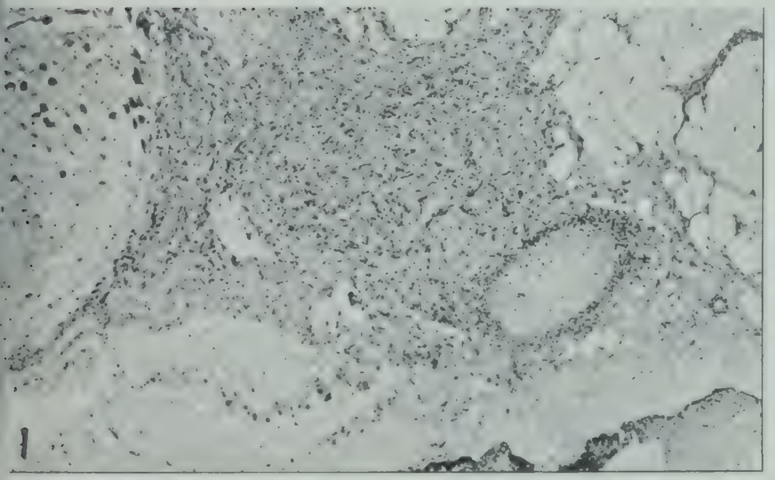

induced
embryo

Fig. 121. Twinning in the chick produced by implantation of organiser.



126. Tissues in a multiple teratoma of the peritoneum.

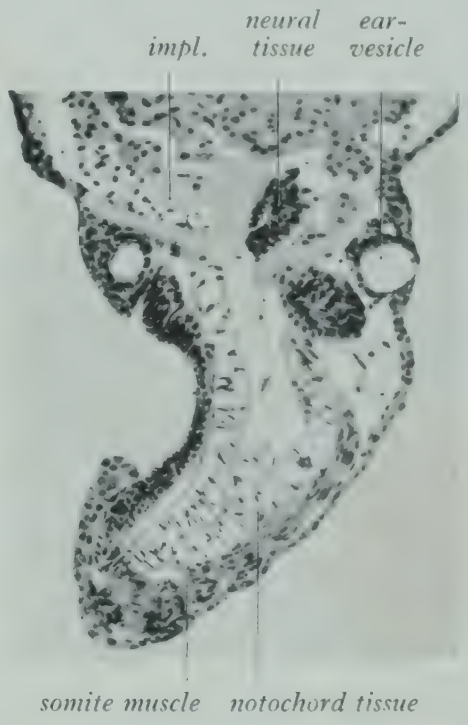

impl.
neural tissue
ear-vesicle
somite muscle
notochord tissue

Fig. 127. Chaotic assemblage of tissues induced in an amphibian embryo by implantation of chick embryo extract into the blastocoele cavity.

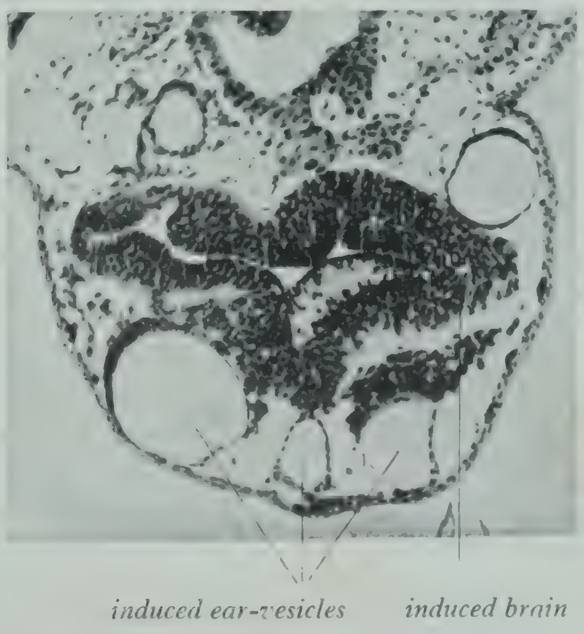

induced ear-vesicles
induced brain

Fig. 128. Numerous ear-vesicles around a brain induced by boiled mouse liver implanted into the blastocoele cavity of an amphibian embryo.

n all this we can dimly see the highly complicated operation of many stimu- ory substances. Evidence has been adduced indicating that the primary aniser of axial structures in the embryo may be of steroid character; it is tain that primary induction can be performed by synthetic hydrocarbons allied the sterols and that similar hydrocarbons are powerfully carcinogenic. When remember that it is likely that carcinogenic substances are produced in the dy by deviations in sterol metabolism, we are prepared to picture that the wild wth of malignant tissues, as well as the highly organised differentiation of uctures in early embryonic development, depend on sterol metabolism for their ntrolling "hormones". In addition, we know that there is a relation between carcinogenic substances and the sex-hormones, so that we can set side by e with this the relatively high frequency of teratomata in the mammalian ovary d testis. The day may come when from a knowledge of the transformations of cyclo-penteno-phenanthrene ring in a given instance, together with an under- nding of what is really meant by the term "competence", we should be able predict just what tissues and morphological forms should arise in a teratoma, at parts of it should be malignant, and how malignant they should be.

The denial that the teratoma is a distorted foetus is important. Budde; cholson; and Willis have all pointed out that no teratoma has ever been found show signs of axiation or metameric segmentation.* Chordal tissue has never en proved to exist in a teratoma. In general the picture is, as has already been d, one of chaotic tissue-formation, implying the presence of all sorts of morpho- netic stimuli *except* the individuation field and its governance of *regional* fferentiation. This is expressed by Willis when he says that teratomata possess "true somatic regions", i.e. masses of nervous tissue but no brain, masses of ne but no bones, renal tissue but no kidney. The condition, indeed, is even ore chaotic than that produced by implanting a dead organiser into a newt bryo, as in Holtfreter's experiments. "We do not accept foetiformity in a gle object", says Nicholson, among those teratomata which have been thought their discoverers to include limbs or vertebral columns. The multiplicity of tain constituents in teratomata is also significant. Willis enumerates cases in ich many separate "nervous systems" were present, and cases in which dozens separate "tonsils" in dozens of separate little patches of "pharynx". Specimens teratomata containing thousands of respiratory or alimentary cysts have been scribed, and the number of teeth may much exceed those normally contained one adult body. We are reminded of the numerous ear-vesicles of Fig. 128. e only possible conclusion is that either the appropriate evocator has been erated in a large number of places or that competence has appeared or been tained in a sporadic chaotic way.

One interesting point that arises here is that just as the muscles and glands of n induced embryo in the amphibia, or of an un-coordinated mixture of tissues duced by a dead organiser, become functional if the host lives long enough,

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The only exceptions are the peculiar "embryonic emboli" or teratoma metastases found in e blood-vessels adjacent to an affected testis (Peyron; Peyron & Limousin). Said to resemble iated blastocysts, they require further critical evaluation.

so the tissues of a teratoma may also be functional. Teratomatous sweat-glan choroid plexus, haemopoietic red marrow, all actively secrete and perform th normal functions. Teratomatous muscle contracts. Particularly significant connection with the remarks just made on sterol metabolism is the fact t patients with testicular and other malignant teratomata often develop a stron positive Zondek-Aschheim reaction (Willis[4]; Crew[2]; Freed & Coppock), whi suggests that the teratomatous tissue may be producing condensed ring compoun similar to oestrone.

Finally, Willis and Nicholson have studied what they call "tissue correlation in teratomata. In a succeeding section we shall study these lower-grade organi effects in normal development. Almost all the smooth muscle in teratomata clearly associated with glandular cavities. The impression is given that t development of the muscle about the gland elements is determined by t presence of the epithelium. Or again, there is a significant correlation betwe islands of cartilage with areas of differentiating nervous tissue. We know from t work of Filatov[1]; A. Luther; and Guareschi[1] that in the amphibia the cartilagino auditory capsule fails to appear if the ear-vesicle has previously been extirpate Conversely, a grafted ear-vesicle will produce a cartilaginous capsule around A similar influence seems to be at work in the teratomata. And stimuli of th kind are undoubtedly involved in all the other processes, tooth formation, orig of meninges, etc.

One class of teratomata can be explained in a particularly straightforward wa from what we know of experimental embryology; namely, the sacro-coccyge tumours known as sacral parasites or sacral teratomata. A drawing, taken from Schwalbe, is shown in Fig. 129. Holtfreter[3] has pointed out that like all the varieties of spina bifida, this condition can be almost certainly referred back to an abnormality of gastrulation. Amphibian exo-gastrulation has already been described (p. 159). Instead of the cells moving in at the blastopore as they should, they will move outwards and away from the ectoderm, so that, if the process is complete, the ectoderm and endo-mesoderm become completely separated. This extreme condition is shown in Fig. 130*a* diagrammatically. The process can occur in all degrees, depending on how long a time exo-gastrulation takes place while the cell stream is moving. Such a series of stages corresponds well with the varying degrees of *Acardius* or *Acephalus* monsters in mammals, where the posterior parts of the body are well formed, but not the anterior and cephalic parts. v. Baer[3] described acephaly in a pig in 1828, and huma cases are always turning up, e.g. the pretty example of le Vine & Wol The amount of neural tissue which is formed in the ectoderm depends exact upon how much it is underlain by the endomesoderm, and if none of th underlying takes place at all, no neural material ever will be formed. No if we compare a mammalian sacral teratoma with the partial exo-gastrulati

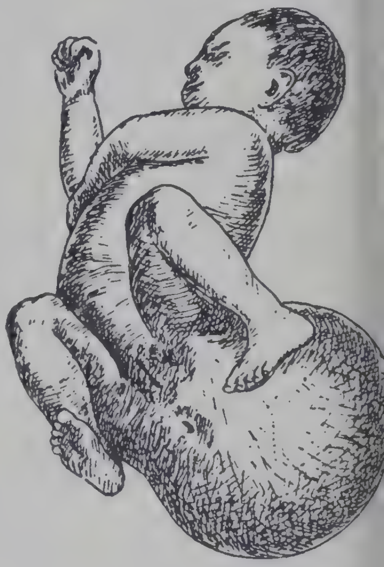

Fig. 129. Human sacral teratoma.

ig. 130*b* we see that there is something of a parallel, for in both cases is an appendage attached in the neighbourhood of the anus, containing riety of tissues. The only important difference is that human sacral tera-ata often contain epidermis and neural tissue. This may be explained by differences between the process of gastrulation in mammalian embryos and in the embryos of amphibia. The contact of endo-mesoderm with ectoderm l that is necessary to produce these neural differentiations. For instance, if ece of competent ectoderm be placed upon the evaginated part of a com-ely exo-gastrulated newt embryo, the evocator will spread to it and induce e tissue and mesenchyme in it (see p. 273).

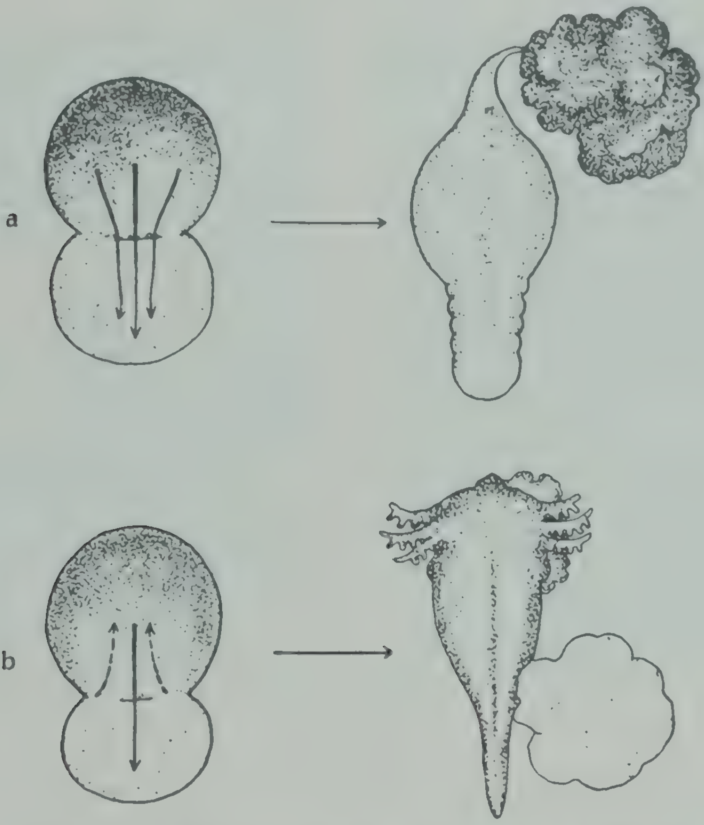

Fig. 130. Diagrams of amphibian exo-gastrulation, to explain the origin of sacral teratomata in man (*a*, complete; *b*, partial).

ntil recently all known cases of teratoma were spontaneous ones. But halovsky and later Bagg[3] have studied the effects produced by injecting 5 % chloride into the testis of the fowl. In many cases teratomata result, some-s so large as to occupy most of the visceral cavity. A typical case is shown in . 131. These tumours may contain cartilage, bone, muscle, fat, connective ue, nervous tissue, glands, epithelium, feather follicles, and lumps of adeno-cinomatous or carcinomatous tissue. The action of the injected metal is ensified by injections of pituitary gonadotrophic hormone, but this alone has such action. Dare we say that the metallic ion has had the effect of restoring embryonic competences at one blow, and that the evocating substances, nally present but functionless in the adult tissue, have then done their work?

Falin, at any rate, in a recent description of such tumours, accedes to this suggesting that presumptive spermatocytes are the responsible cells. experimental production of teratomata opens up all kinds of valuable possibi

In a later paper, Bagg[4] found that if autolysed testis tissue is injected a same time as the zinc chloride, a teratoma develops subsequently at the fo site of injection, not the latter. The metal itself appears to be eliminated qu from the testis.* Michalovsky-Bagg teratomata are transplantable to other (Anissimova).

Perhaps these facts are connected with the curious phenomena of "seminc (Champy, Lavedan & Marquez; Kirschbaum & Jacobs). If the left testis of a

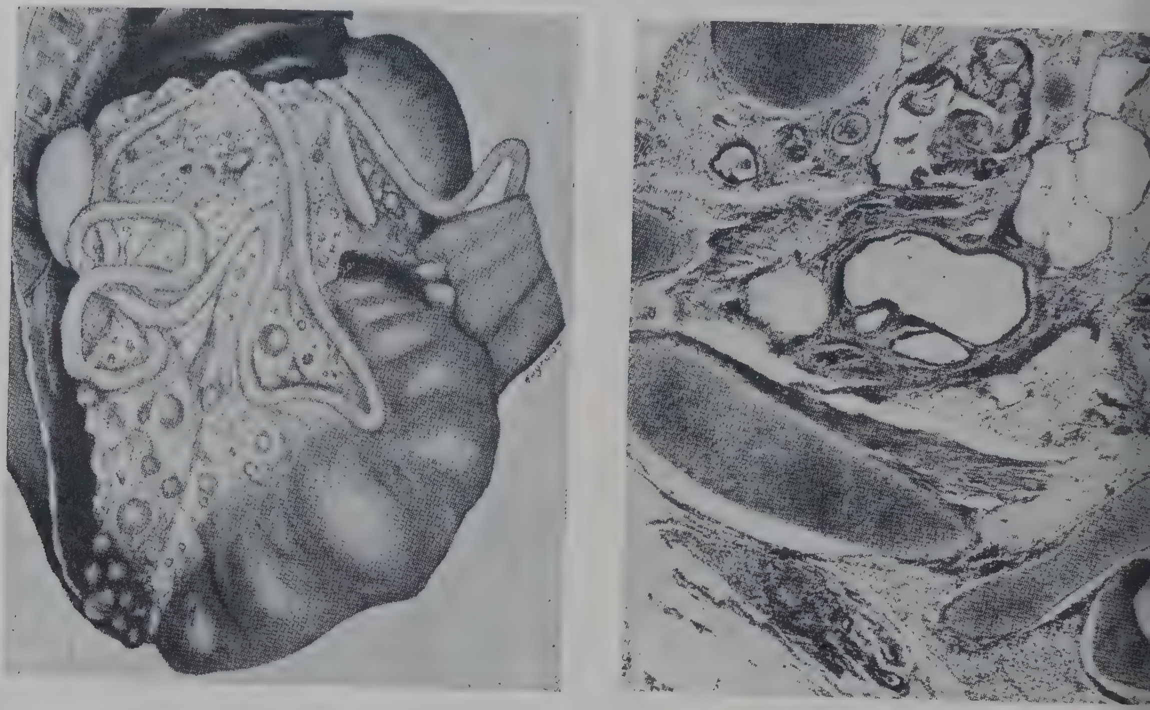

Fig. 131. Experimentally produced teratoma of the testis in the fowl.

is removed, the atrophic right one will undergo compensatory hypertrophy, often this is overdone and a characteristic tumour of large size results. In s over-regeneration there is thus an escape from the individuation field.

This section shows how important is evocator control in normal developme Appearance of supernumerary evocators may be a normal process, as in some s of polyembryony, or it may be a pathological one. If the primary organise concerned, the result will be a complete twin or a complex monster, if second- third-grade organisers are concerned, the result will present itself to the mor anatomist as a teratoma. In this latter case we have also to consider the persiste or recuperation of competence in tissues whence it should have disappeared, it is clear that the possible permutations and combinations of irresponsi organisers and perversely competent tissues will provide everything that contained in the literature on teratomata; the "foetiformity" theory notwi ling. Pathologists have recently begun to apply this point of view successfully rious specialised directions, e.g. multiple meningeal and perineural tumours rster-Drought, Carnegie-Dickson & McMenemey); odontomata (Sprawson); gn ovarian teratomata (Willis[4]), the rare chordomata (Richards & King), etc. ther phenomena besides teratomata suggest the anomalous appearance of petence where it ought not to be. In the abnormality nsects, sometimes of genetic origin, known as *thetely*", a caterpillar may form pupal antennae dschmidt[5])—see Fig. 132—or pupal wings (Ferla). Goldschmidt[8] (*Physiological Genetics*, p. 173) ests that as the development of the imaginal discs der hormonic control, the threshold may have been naturely lowered. This is perhaps another way of ng that competence has appeared where it should not done. The converse of this is seen in insect inters, where competence seems to be unduly damped, the result that the development of the imaginal discs ns extremely late. As chitinisation of the soft parts he end-stage in insect development, these organs ome chitinised as imperfect stages or even as imaginal s instead of completed organs. Hence the embryonic cture becomes "fossilised" and appears in the adult ldschmidt[4]). This subject will be taken up again in the section on deteration in insect development and metamorphosis (p. 466). We have now to sue further the malignancy problem.

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* It may be remembered that zinc is used as a protein precipitant. Would other denaturing age have similar effects? Other zinc salts are active (Falin & Gromzeva).

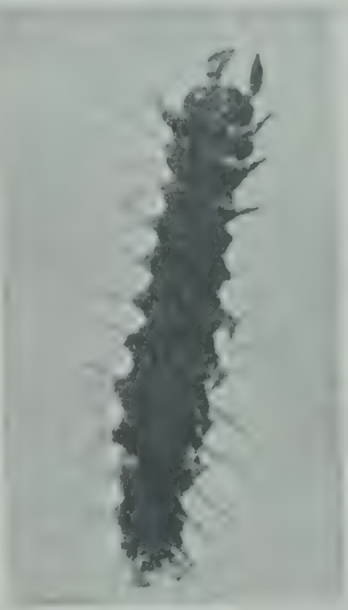

Fig. 132. Prothetely.

·38. Organisers and cancer

2·381. The escape from the individuation field

he foregoing discussion has brought us to the verge of the problem of gnancy. In normal development, differentiation and growth are highly nised. In the last section, a short review of the problem of teratomata showed t happens when differentiation proceeds in an unorganised way. We are rtunately only too well acquainted with circumstances in which growth also mes unorganised, and certain types of cell, proliferating in superabundance, de the territories of peaceful tissues performing their specified normal tions.* At first sight organiser phenomena have little to do with cancer, for ne thing is more characteristic than any other of evocator substances, it is they are concerned with the stimulus to a specific differentiation, not a eral growth.† But evocation, as has already been pointed out (p. 125),

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he growth rate of neoplasms is quite comparable with that of embryos (Schrek; Sugiura & edict).

his is well expressed by Fischer-Wasels: "Das Wachstum der malignen Geschwulst unter- idet sich prinzipiell und wesentlich von dem Wachstum der embryonalen Zelle. Die Schnellig- der Zellbildung ist nur ein äusserliches Merkmal. Das Wesen des malignen Wachstums gen, das nämlich irgendeinem höheren Plane, insbesondere dem Organisationsplane des nismus, nicht unterstellt ist, findet sich bei der Embryonalzelle nicht, ja wir finden hier

is only the half of organisation. The organiser has a certain relation to th of the whole body which we may speak of as existence in an individuation The main characteristic of an individuation field is that all tissue lying wi tends to be built up into one complete embryo, and in any one part of th all tissue tends to be built up into the organ corresponding to that part. of the most important aspects of the cancerous growth is that it is an *escap* this controlling pattern or field.* This manner of stating the problem may i be rather fruitful.

It is at once evident that a great deal will depend on the extent to whic individuation field (the assumption of which is indispensable in embryo *persists into the adult condition.* It may be suggested that power of regene is a suitable measure of this. If so, the mammalian individuation field has wholly lost during development. Nevertheless there are organisms which p remarkable powers of regeneration in the adult state, such as the newt which so much experimental work has been done (cf. Section 2·71). I adult newt, the leg area is still so potent that it can mould into a limb any of competent tissue either grafted into it, or formed on it as a regeneration We are led at once to ask whether there is any difference in susceptibili cancer between Urodeles which can regenerate lost limbs and tails so well, Anura and reptiles which have no such capacity. Unfortunately, it seems this is not known. Cramer[1], in his review on cancer in animals, points out the relatively short lives of many animals preclude the clear appearanc many tumours which may have made a beginning. It may perhaps be of s ficance that a search through the literature on amphibian cancer gives far cases of neoplastic growth reported for reptiles and anurans than for uroc As the data have not so far been assembled, a list (as complete as possib given in the accompanying table (Table 21). The cases are unfortunately to in number to allow of any statistically reliable conclusions; for example, it be remembered that for many years past the frog has been a much more com laboratory animal than the newt. In illustration of the table, I give two pict the first, from Eberth's classical paper of 1868, shows the external appearan a frog suffering from multiple nodular adenomata of the skin (Fig. 133); the se shows a cross-section of Murray's carcinomatous newt[1] (Fig. 134). It m observed that the ectodermal tissues are alone affected, and that there i infiltration of the underlying muscle.

Perhaps the best-known amphibian tumour is the carcinoma in the leo frogs of New England (*Rana pipiens*), described by Lucké[2] and Lucké & Sch berger. The kidneys of some 2 % of the population show non-encapsu

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sogar das grossartigste Ineinandergreifen kompliziertester Gestaltungsvorgänge, das wir i Natur kennen. Alles embryonale Wachstum und Geschehen ist beherrscht von dem Orga tionsplan des Körpers, von der Einheit des Individuums. Also eine wesentliche Ähnlic der beiden Wachstumsformen ist überhaupt nicht vorhanden" (p. 1388). We may leave o account the many speculations (such as may be found in the pages of W. R. Williams) on the c of cancer from "embryonic cell-rests", etc. Fischer-Wasels discusses them at length, an recent progress along biochemical lines has deprived them of most of their interest.

* A morphological escape. Histologically and functionally, of course, tumours are quite fai to the tissues from which they take their origin.

Author	Date	Species	Nature of tumour		No. of cases	Remarks
			Class	Site		
REPTILES						
Bland-Sutton, J.[1]	1885	Indian monitor lizard	Chondroma	Limb	1	
		Python sebae	Carcinoma	Ovary	1	
Pettit, A. & Vaillant, L.	1902	*Python* sp.?	Fibroma	Stomach	1	
Pick, L. & Poll, H.	1903	Brazilian tortoise, *Platemys geoffroyana*	Benign tumour	Thyroid	1	
Koch, M.	1904	Lizard, *Lacerta agilis*	Papilloma	Occipital skin	1	
Plimmer, J.	1912 & 1913	Tortoises	Carcinomata	?	2	2 in 6000 autopsies
Schwarz, E.	1923	American lizard, *Tupinambis teguixin*	Carcinoma	Hand	1	No metastases; no infiltration
Scott, H. H.	1927	*Crocodilus porosus*	Sarcoma	Liver	1	Metastases
Anonymous	1930	*Crocodilus* sp.?	"Non-cancerous"	?	1	
Smith, G. M. & Coates, C. W.	1938	*Chelonia mydas*	Fibro-epitheliomata	?	4	
ANURANS						
Eberth, C. J.	1868	Frog	Multiple adenomata	Skin	Many	
Smallwood, W. M.	1905	Frog	Columnar-celled carcinomata	Adrenals	1	
Plehn, M.	1906	Frog (*R. esc.*)	Multiple carcinomata	Ovary	1	No infiltration
Murray, J. A.[1]	1908	Frog	Adeno-carcinomata	Skin of thigh	2	No metastases
Carl, W.	1913	Frog (*R. esc.*)	Hypernephroma	Adrenals and kidney	1	
Pentimalli, F.[1]	1914	Frog	Adenoma	Skin	1	
Secher, K.	1919	Frog	Cystadenoma	Skin	1	No penetration of deeper layers, no metastases
Lucké, B.[2]	1935	Frog (*R. pip.*)	Adeno-carcinomata	Kidneys	300	Infiltration, no metastases
URODELES						
Pettit, A. & Vaillant, L.	1902	Salamander, *Cryptobranchus* sp.?	Fibropapilloma	Hand	1	
Pick, L. & Poll, H.	1903	*Cryptobranchus japonicus*	Carcinomatous cystoma	Testis	1	Metastases
Murray, J. A.[1]	1908	Newt, *Triton cristatus*	Nodular carcinoma	Skin of head and tail	1	No infiltration
Schwarz, E.	1923	Salamander, *Megalobatrachus maximus*	Fibroma	Hand	1	
Champy, C. & Champy, C.	1935	Newt, *Triton*	Epitheliomata		Many	

NOTE. Teutschlander mentions a melanosarcoma of axolotl in a table but there is no reference to it in his text. Whether one of the above cases is meant must remain uncertain.

adeno-carcinomata infiltrating and destroying the surrounding renal tissue, often attaining a size so large as to cause much displacement of the abdo viscera. Metastases are rare, but the tumour is transmissible experimentall

It is obvious that opportunities for experimental attack along these line inviting and numerous. Unfortunately amphibian tissue seems to be exceptio refractory to carcinogenesis by hydrocarbons (Duran-Reynals; van Heyning though success has been achieved, both in the adult newt (Koch, Schreib Schreiber) and the frog tadpole (Briggs[2]). But if amphibian cancerous ti transplantable on to newt tissues could be found, it would be possible to whether such wildly growing material could be mastered by the individua field controlling a limb area. If so, it should be organised into a normal li Enquiry could also be made into the degree of competence still possessed by s cancerous tissue. By subjecting it *in vitro* to the action either of a living organ or of evocator substances, its potentialities might be revealed. The influenc carcinogenic agents on regeneration is also an obvious direction of study. H experiments have again so far been negative; thus Martella painted tar upon skin of the newt *Molge* near the tail without any effect, Tokin found no influe of the synthetic carcinogenic hydrocarbons which he tried on the regeneratio *Hydra*, and Slizyński obtained equally negative results when painting tar on *Dr phila*. One might picture that if the powerful growth stimulus of a carcinog

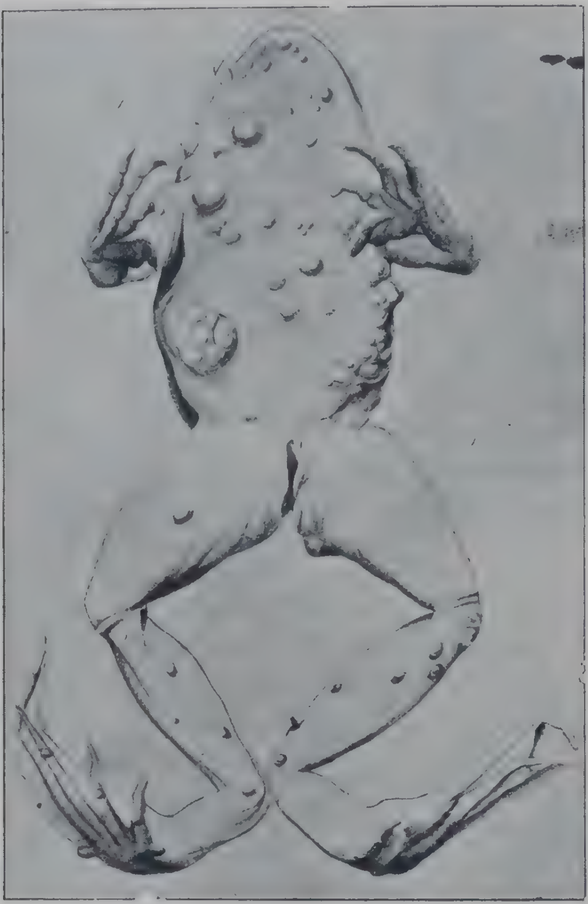

Fig. 133. Multiple nodular adenomata in the frog.

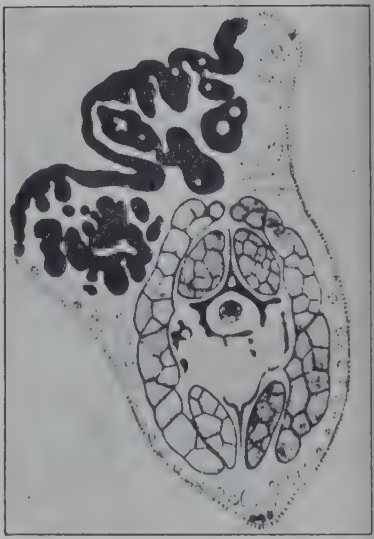

Fig. 134. Carcinoma of the tail in the newt.

stance acted within a powerful individuation field, the structures produced ıld show some sort of harmoniously regulated differentiation.

'he nearest approach to a verification of the idea expressed in this section urs, perhaps, in Pflugfelder's remarkable work on the stick insect, *Dixippus* *osus*. If the *corpora allata* (see p. 465) are removed, the regeneration of endages after amputation or autotomy no longer follows a normal course, ng rise instead to all kinds of wild, and even teratomatous, proliferations. l more extraordinary, the structure which appears after excision of the :ted eye is an ocellar type of eye which this species never normally possesses, ugh other phasmids do (cf. the balancer-sucker situation, p. 342). The mones of the *corpora allata* in this insect may therefore have a profound effect h on the individuation field and on the competences of the blastema cells. e further investigation of the whole question seems to promise much.

n a succeeding section (p. 430) we shall have something to say about regeneraı phenomena as a whole. From the foregoing line of thought it follows that eneration tissue ought to show some of the properties of embryonic tissue, ripotence, competence, etc. That it does so has been widely believed, but the ıl proof appears hardly yet to have been given. As we shall see, lens comence, at any rate, has been shown to be present in the regenerating blastema hotté & Hummel); and Schotté[5] has stated that he and his collaborators have ained inductions of ear-vesicles by brain grafts in the regenerating tail tissues adpoles. More still, he reports that implantations of pieces of mouse embryos o mouse fibrosarcomata led to teratomatous arrays of organised structures. e full publication of these results will be awaited with interest.

2·383. Evocators, carcinogens and oestrogens

The second way in which embryonic evocators are connected with the problem cancer is due to the fact that synthetic carcinogenic substances have been found ct strongly as primary evocators in the amphibian embryo (Waddington & :dham; Waddington[21]; Shen[1,2]), cf. pp. 178 ff. This necessitates a discussion of biological effects of substances of steroid and polycyclic hydrocarbon character. vill be brief, since there exist many valuable reviews of this literature.

Ve shall begin with the chemical carcinogens.* In 1910, when the researches v to be described began, it had long been known that manual workers who ıe into contact with tar, especially when originating from gasworks, were ticularly liable to suffer from cancer. The credit for demonstrating that cancer ıld be produced experimentally in animals by painting tar or other chemical ›stances on the skin is usually ascribed to Yamagiwa & Ichikawa, who in 1915 tained cancerous growths in the ears of rabbits by long-continued applications tar. Some years earlier, however, Bayon had mixed a watery extract of gas›rks tar with sterilised lanoline, and, on injecting this into the rabbit's ear, tained enormous epithelial proliferations simulating cancer. These experiments

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The term "carcinogen" is here loosely used, but it is common to speak of the chemical cinogens as producing sarcomata if injected intra-muscularly, or epitheliomata if painted on skin. Some compounds (e.g. 10-methyl-1:2-benzanthracene) produce only the former, others ;. 9:10-dimethyl-1:2-benzanthracene) only the latter (Badger *et al.*).

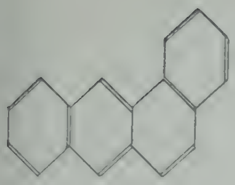
are now somewhat difficult to evaluate, since although Bayon demonstr negative controls with lanoline alone, there is reason to believe that lanoline sometimes contain carcinogenic substances (Peacock[1]), and moreover the carc genic substances of tar are not themselves water-soluble. Bayon's work, howe has historical interest.

The two Japanese investigators were soon followed by two Swiss, Bloc Dreifuss, who showed that the carcinogenic activity was due to neutral nitrog free substances possessing a high boiling-point, and the fractionation of tar t began in earnest under the aegis of Kennaway, Hieger, Cook, and their co borators in London. Isolation of the active fractions was facilitated by Mayneo discovery that the fluorescence spectra of carcinogenic tars differed from th of non-carcinogenic tars. The first carcinogenic hydrocarbons prepared from in a pure state were described by Cook, Hewett & Hieger, and later they w synthesised in the laboratory (Barry, Cook, Haslewood, Hewett, Hieger Kennaway). They all contained the tricyclic phenanthrene condensed r system, similar to that of the long-known animal and plant sterols. The m active of these substances is 3 : 4-benzpyrene, but 5 : 6-cyclopenteno-1 benzanthracene is also very efficient. These produce squamous-celled carcinom in rodents and even metastases are not uncommon. 1 : 2 : 5 : 6-Dibenzanthrac is less efficient than the two foregoing compounds in producing carcinomata, when injected subcutaneously into rodents it gives rise to sarcomata. Nitrog may be introduced into the ring skeleton, as in 1 : 2 : 5 : 6-dibenzacridine 3 : 4 : 5 : 6-dibenzcarbazole, without abolishing the carcinogenic activity. One the parent hydrocarbons of all these substances, chrysene, has been claimed be active, but this is uncertain, and its activity cannot be very marked (Barry Cook). It often contains 3 : 4-benzpyrene as an impurity (Schürch & Winterstei

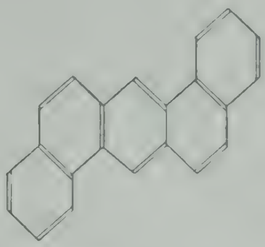
The following description of chemically induced cancer is given by Murra If a mouse is painted on the back twice weekly the hair is first destroyed and covering epithelium of the skin thickens, the lower layers showing an infiltrat with lymphocytes, mast cells, and polymorph leucocytes. Then discrete wa prominences appear with the histological structure of benign papillomata. If painting is now stopped (15 weeks) these warts may regress and drop off, bu the substance painted has been highly active they will now grow more and m rapidly, adhere to the deeper tissues, which will become infiltrated, and metastas into the adjacent lymph nodes, the lungs and sometimes into other organs.

This description recalls the conception of progressive determination us in experimental morphology. Many other causes which produce uncontrol cancerous growth are of course known; the secretions of some animal parasit the application of cold and of X-rays, may be mentioned. But in spite of pleas to the contrary, the simplest working hypothesis seems to be that these l precisely known carcinogenic agents act by liberating within the tissues substan more or less closely chemically related to the carcinogenic hydrocarbons isola from tar. A number of interesting facts are relevant to this.

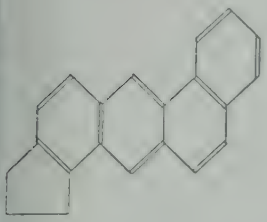
Early in the development of the subject it was found that carcinogenic su stances were produced by exposing yeast and human skin to high temperatu (e.g. 920° C.), with the formation of tars (Kennaway). This showed that su substances could be formed from chemical compounds normally present in living tissues. But later on a more elegant demonstration of this was given Cook & Haslewood and by Wieland & Dane, who found that it was possib starting from a sterol compound (the bile acid, desoxycholic acid), to synthes through various intermediary stages (12-keto-cholanic acid and dehydro-n cholene) one of the most strongly active carcinogenic hydrocarbons know



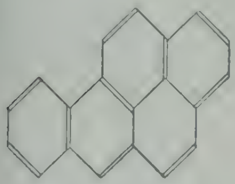
1 : 2-Benzanthracene



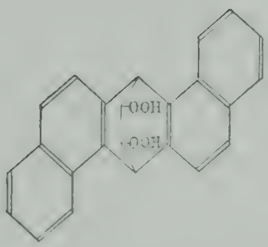
1 : 2 : 5 : 6-Dibenzanthracene (C)



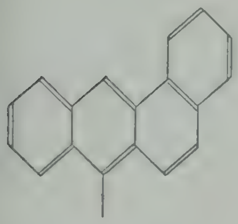
5 : 6-Cyclopenteno-
2-benzanthracene (C, O)



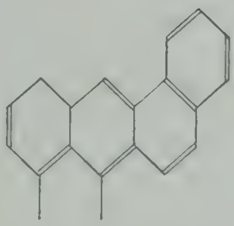
: 4-Benzpyrene (C, E, O)



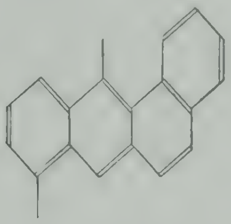
1 : 2 : 5 : 6-Dibenzanthracene,
endo-α-β-succinate (C, E)



10-Methyl-
: 2-benzanthracene (C)



5 : 10-Dimethyl-
1 : 2-benzanthracene (C)



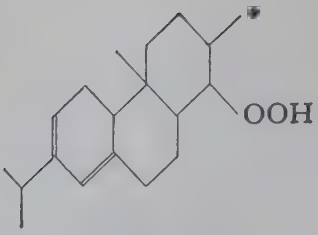
5 : 9-Dimethyl-
1 : 2-benzanthracene (C)

as powerful as methylcholanthrene

In the accompanying tables of formulae abbreviations are used as follows: C, carcinogenic;), oestrogenic; E, active as primary evocator.

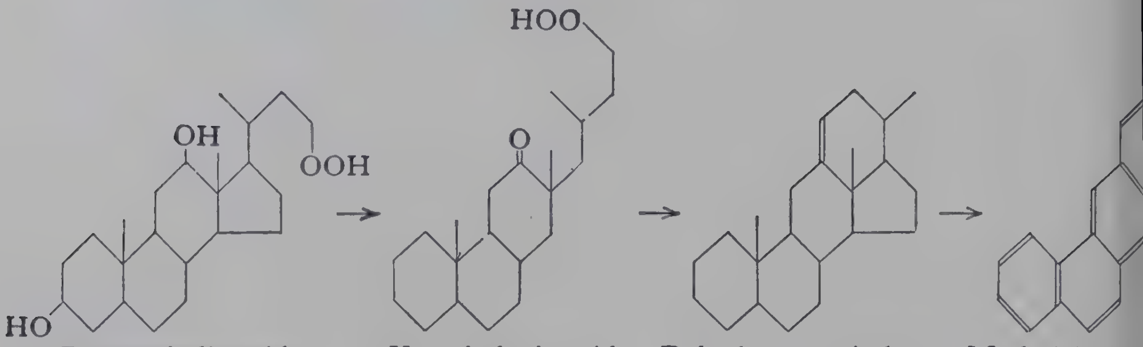
Reviews on the Steroids

al Review: Kühn[2]
s and related compounds: Fieser[1]; Lettré & Imhoffen; Rosenheim & King; Sobotka[2]; Vindaus[2]; Schoenheimer & Evans; Mozołowski
cids: Sobotka
in D: Bills; Brockmann; Friedmann[2]; Rominger
heart poisons: Jacobs & Elderfield[2]; Elderfield; Tschesche[2]
heart poisons: Fieser[1]; Tschesche[2]
nins: Fieser[1]; Tschesche[2]
oids: Fieser[1]
sex-hormones: Ruzicka[1]; Dodds; F. C. Koch
ale sex-hormones: Dodds
nal cortex hormone: Wintersteiner & Smith
ogens: Dodds; Dodds & Lawson[3]; Schmidt-Thomé; Pincus & Werthessen[3]
nogens: Cook[3]; Fieser[1, 3]; Cook, Haslewood, Hewett, Hieger, Kennaway & Mayneord; Cook & ennaway; L. Loeb[5]
erpenes; Ruzicka[2]; Jacobs & Elderfield
nol: Tschesche[1]

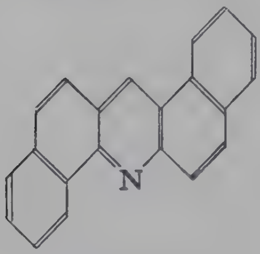


* Position of this methyl group uncertain.

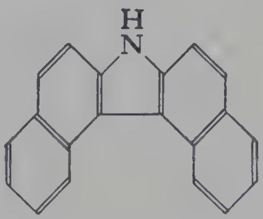
Abietic acid


OH
OOH
HO
→
HOO
O
→
→

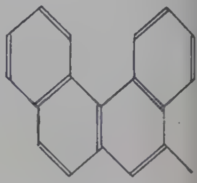
Desoxycholic acid 12-Ketocholanic acid Dehydro-nor-cholene Methylcholan
(C, E)



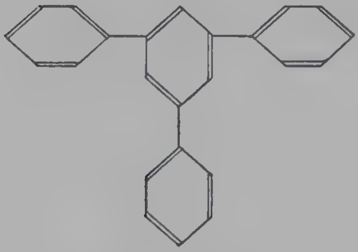
1 : 2 : 5 : 6-Dibenzacridine (C)



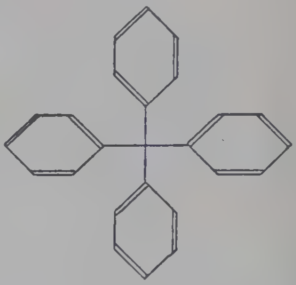
3 : 4 : 5 : 6-Dibenzcarbazole (C)



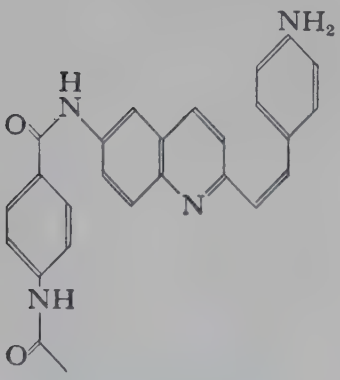
2-Methyl-
3 : 4-benzphenanthren



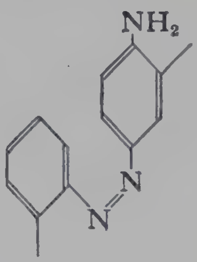
Triphenyl-benzene (C)



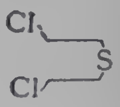
Tetraphenyl-methane (C)


NH$_2$
H
N
O
N
NH
O

Styryl blue (C, E)
[2-(*p*-aminostyryl)-6-(*p*-acetylamino-benzoylamino)-quinoline methoacetate]



2-Amino-5-azo-toluene (C)



Dichlorethyl sulphi
(anti-C)

ylcholanthrene. It was subsequently found by Ghiron and by Cook, Kenna-
& Kennaway that desoxycholic acid itself has a slight carcinogenic activity.
his indicates, though of course it does not prove, that from sterols normally
rring in the body, a deranged metabolism may produce specific carcinogenic
tances which will stimulate certain cells to uncontrolled proliferation (Cook[1,2]).
reliminary work Burrows & Mayneord and Polletini have obtained cancer from
-ray irradiation products of cholesterol and ergosterol. Bergmann[2] suggests
the side-chain of cholesterol might participate directly in a cyclisation.

rthermore, it has been found that polycyclic carcinogenic hydrocarbons are
ised photochemically to give water-soluble products (Allsopp). These products
an inhibitory action upon important respiratory enzymes such as lactic
drogenase, and roughly speaking the action is greater the more active the
ocarbon is in cancer production (Boyland[2]). This action resembles that of
nious acid, a substance which is known to be carcinogenic, so that we may
sage the following classification of substances: (*a*) the polycyclic hydrocarbons
nselves, (*b*) substances (or influences) which stimulate the tissues to produce
r own polycyclic carcinogens, (*c*) substances of very divergent chemical
position which perhaps stimulate the cells or inhibit processes within them
point different to that affected by the polycyclic carcinogens. It has also been
vn that the oxidation products of the active hydrocarbons inhibit both
iration and glycolysis in every tissue investigated (Boyland & Boyland[1]). In
way we begin to see how work on the chemical stimulating mechanism may
up with work on the metabolic peculiarities of malignant as opposed to
nal tissue, already pushed so far by the school of Warburg (cf. *CE*, p. 758).

he reviews of Cook[3]; Cook, Haslewood *et al.*; Cook & Kennaway and Loeb[5],
the discussion opened by Murray[2], give full details of the chemistry of the
pounds so far found to be carcinogenic. Fieser[1,2,3] has reviewed the relation
veen chemical structure and function. But it is now clear that the condensed
system is not indispensable in chemical carcinogenesis. Morton, Clapp &
nch found symmetrical triphenyl-benzene and tetraphenyl-methane to be
lerately active.* Similarly Browning, Gulbranson & Niven showed that the
styryl blue, which contains one heterocyclic ring as well as three benzene
, was strongly carcinogenic. Even a compound with only two rings may be
e, such as the 2-amino-5-azo-toluene of Shear and Kinosita, which produces
carcinomata. The effects produced by styryl blue on respiration and
phorylating glycolysis have been studied in interesting work by Pourbaix.

he relationships between the many ring compounds of biological activity will
me clearer if we glance at their formulae. Those who wish to investigate any
t in further detail will find what they want in the excellent monographs of
er[1] and of Lettré & Imhoffen.

he parent hydrocarbons of the whole series are anthracene, phenanthrene,
chrysene. Phenanthrene, isomeric with anthracene, was discovered by Fittig
stermayer in 1872 in tar. But if these are the structural parents, cholesterol
f is the starting-point of our knowledge of the sterols. A constituent of all
nal tissues and cells, it occurs in particularly large amounts in brain, adrenal
ex, and bile, and has been known since the eighteenth century and the days
hevreul and Thudichum. It has undoubtedly a large part to play in the cell
cture (see p. 663). It is synthesised by animals. The sterols of plants, ergosterol
stigmasterol, differ by having a double bond in the side-chain and (in the
ner) ring II more aromatised; they are readily synthesised in the plant but not
h absorbed from the intestinal tract of animals (Schoenheimer[1]). It is inter-

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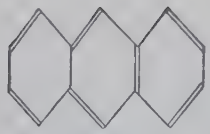
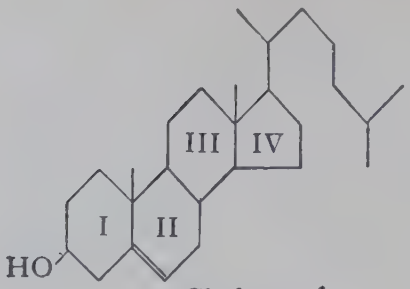
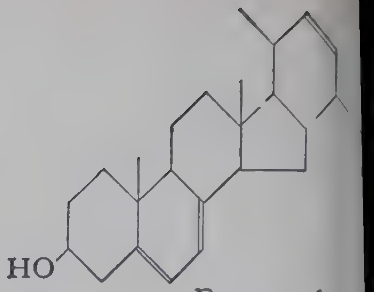
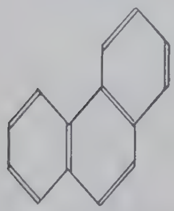
his is contested by the London school (Bachmann, Cook *et al.*; Badger, Cook *et al.*).

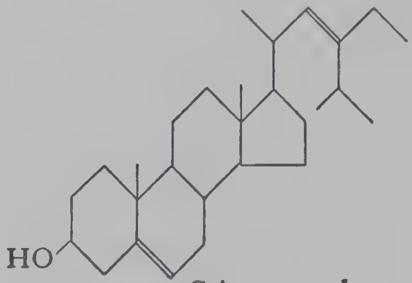

Anthracene


I
II
III
IV
HO
Cholesterol

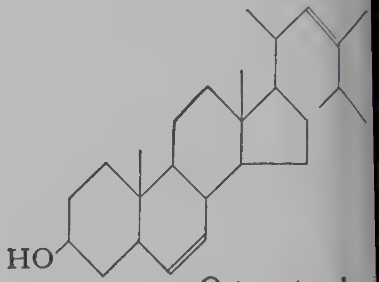


Ergosterol

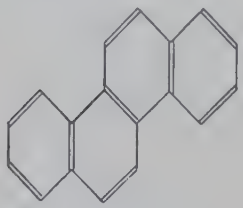

Phenanthrene

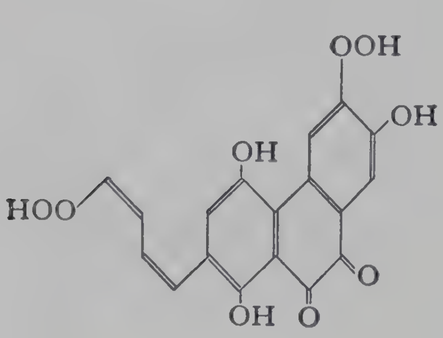


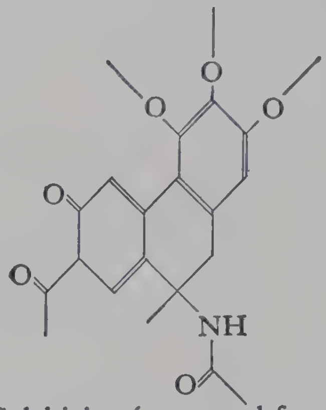
Stigmasterol



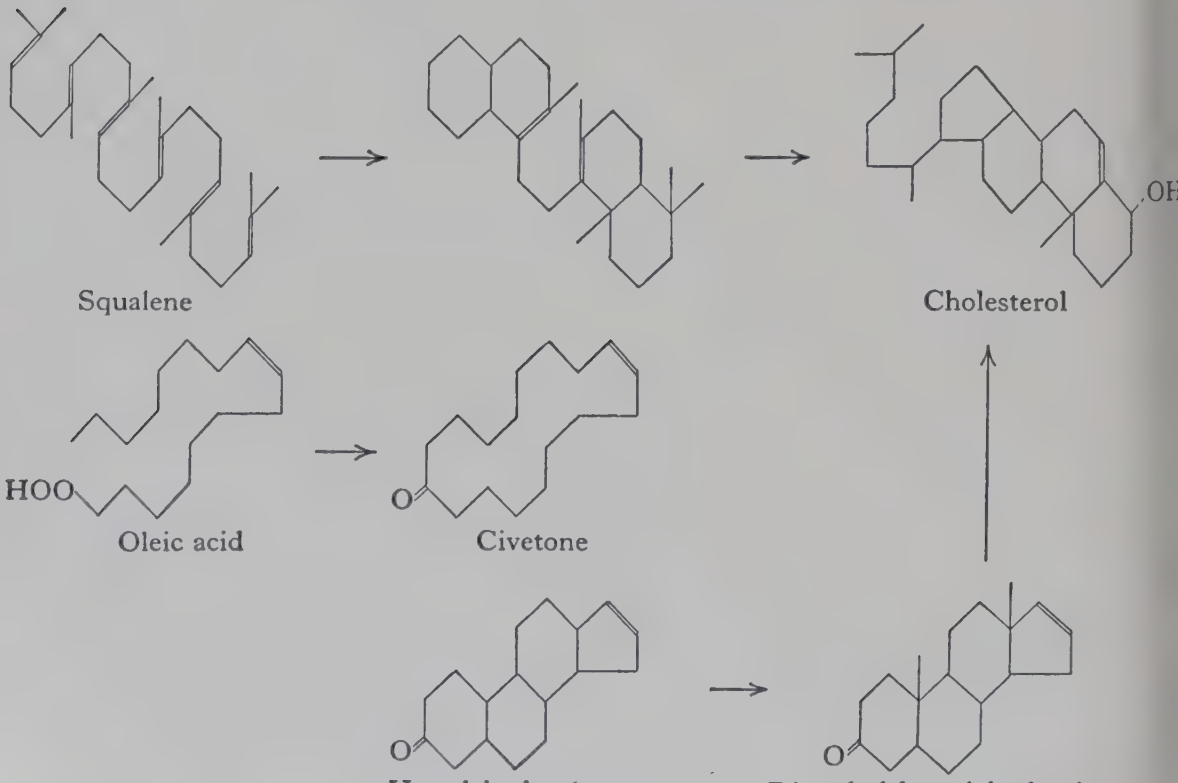
Ostreasterol


Chrysene


OOH
OH
OH
HOO
OH
O
O
Telephoric acid



Colchicine (suggested formula)



Squalene

Cholesterol

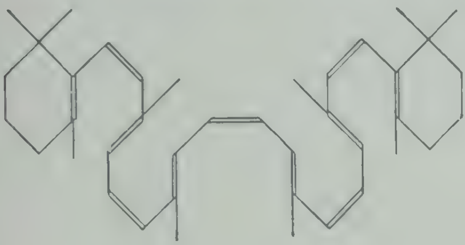
Oleic acid

Civetone

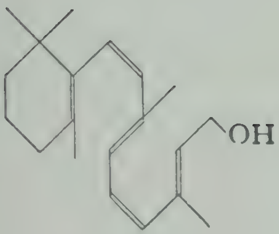
Hexadehydrocivetone

Dimethyl-hexadehydrocivetone

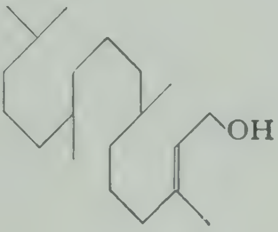
ng that among the invertebrates are found sterols, such as ostreasterol from oyster (Bergmann[1]), which resemble those of plants in having a double bond he side-chain, but are no more aromatised in ring II than the animal sterols. e phenanthrene ring occurs more widely than the sterol ring system; thus the ious orange pigment isolated from certain moulds, telephoric acid, has a nanthrene structure; and so also the drug colchicine, which has a powerful ibiting action on cell-division, arresting it in metaphase (Brues & Cohen; land & Boyland[3]; see p. 509). Many alkaloids, too, such as morphine, codeine, l thebaine, possess the phenanthrene ring system.



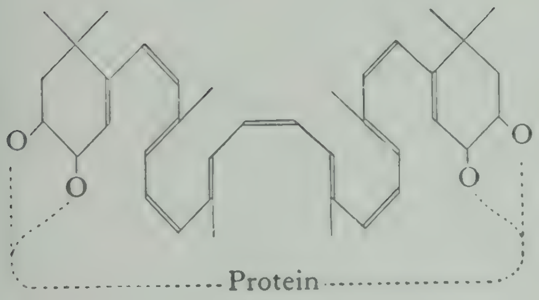
β-Carotene



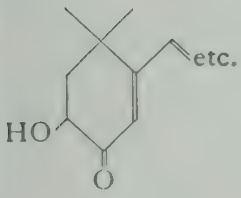
Vitamin A



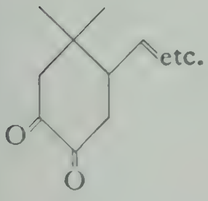
Phytol



Ovoverdin (green)



Astaxanthin (the naturally occurring red carotenoid)



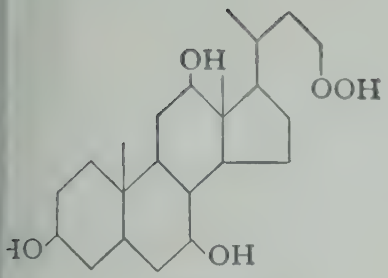
Astacin (formerly thought to be the naturally occurring red one)

Nothing certain is known regarding the mode of synthesis of the sterols, nor e nature of the substances to which they are most closely related. Some have agined a connection with the polyterpenes. Thus the non-cyclic polyterpene, squalene, an oily substance present in large amounts in the livers of cert elasmobranch fishes, has been transformed by cyclisation into tetrahydrosqual which bears a certain relation to cholesterol, though rather a distant one (Ka & Helfenstein), and a tetracyclic triterpene into a pentacyclic triterpene (Beyn Heilbron & Spring). If this relationship existed, there would be a connect between the steroids and the lipochrome pigments, which are all polyterpen such as carotin, astacin (cf. pp. 210, 652), vitamin A, and the alcohol phy attached to the tetrapyrrol-magnesium framework in chlorophyll (cf. the revi of Karrer[2]). Physiological experiments, such as those of Channon, which h essayed to prove this relationship, have not yet quite succeeded in doing Others have imagined a connection between the sterols and the higher fatty aci and certainly oleic acid can be cyclised into civetone, which could then giv sterol ring system through hexadehydrocivetone and dimethyl-hexadehyd civetone (Windaus). In plants the sterols may be synthesised from carbohydrat if the interesting schemes of J. A. Hall really occur in nature.

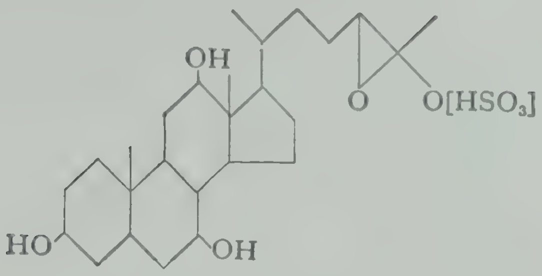
Another class of steroid compounds are the bile acids, such as cholic ac (reviewed by Sobotka[1]). Combined with glycine or taurine, they are secreted the liver in all vertebrates. They are characterised by having at least two hydro groups on the ring system, and a carboxyl group on the side-chain. In the b of lower vertebrates, substances intermediate between the bile acids and oth steroids occur, such as the interesting compound scymnol from elasmobran bile, which has no acid group, a long side-chain, and a sulphuric ester groupi terminal to it.

Anti-rhachitic compounds form still another class. The well-known irradiati of ergosterol (reviewed by Bills and by Friedmann[2]) leads to vitamin D_2, in whi the side-chain is unaltered, but the ring system has broken at ring II. The natur vitamin, D_3, is identical with this save that it has the side-chain characteristic cholesterol. For details of the action of this and other vitamins mentioned, t monographs of L. J. Harris[2] will of course be consulted. But other compoun besides the natural D vitamins themselves possess minor degrees of anti-rhachi potency, e.g. the irradiation product of 7-dehydrositosterol.

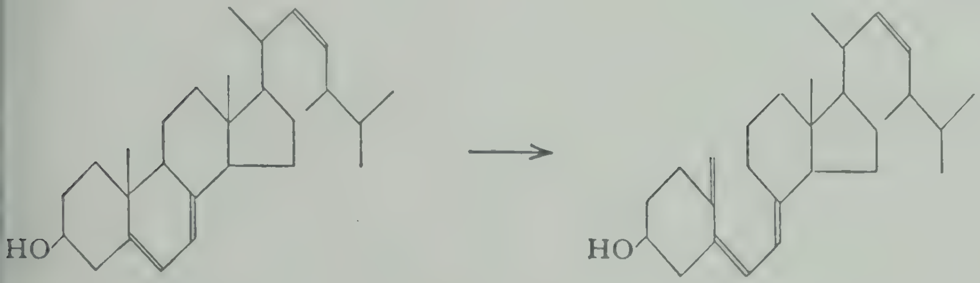
It has been one of the triumphs of organic chemistry in recent years to sho that other, more obscure, substances of great biological activity also possess t sterol skeleton (see the review of Tschesche[2]). Plants having an action on t heart have been known for many centuries, and an extract of *Strophanthus hispid* is used as an arrow poison by primitive tribes. Foxglove was known to mediaev medicine. Digitalis, the "sheet-anchor of cardiac therapy", was introduced in the modern pharmacopoeia by Wm. Withering in 1785. The active principles plant heart poisons consist of sterols united with sugars, mainly methylpentose Digitoxigenin is typical. The hydroxyl on ring I is placed similarly to that cholesterol, but there is an additional one at the junction of rings III and I and in the side-chain there is an unsaturated lactone ring. Strophanthidin unique in having an aldehydic group instead of the usual methyl group betwe rings I and II. Oxygen in the side-chain is also characteristic of the poisons toad venom, known from remotest antiquity and possessing pharmacologic properties similar to those of the plant cardiac glycosides; hence the ancie Chinese drug, *ch'an su*, prepared from toads. Many species of *Bufo* conta bufotoxin in skin-glands, a compound of a fatty acid, suberic acid, arginine, a the steroid bufotalin. Here the heterocyclic ring in the side-chain is six-, n five-membered. The saponins, too, are of steroid or polyterpene nature. Plan which give soapy or foamy extracts have been known for centuries (cf. comm names such as soapwort) and are often used for washing purposes, as for instan



Cholic acid

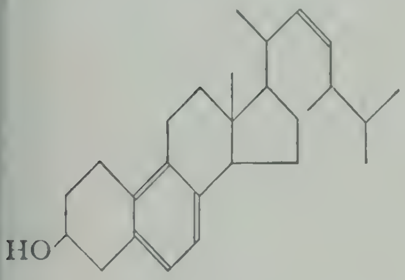

OH
O
O[HSO₃]
HO
OH

Scymnol

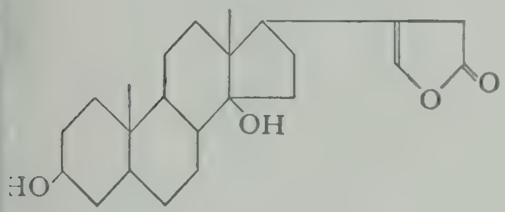


Ergosterol

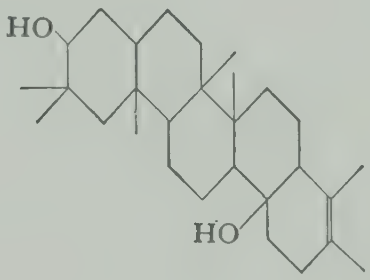
Vitamin D_2



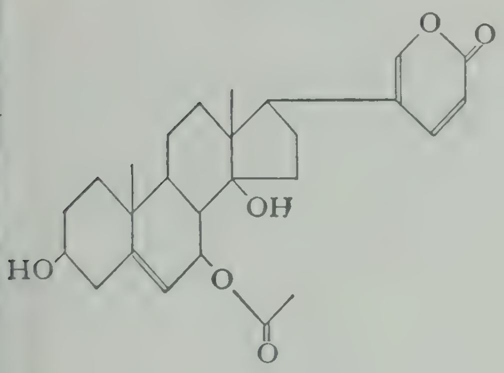
Neo-ergosterol



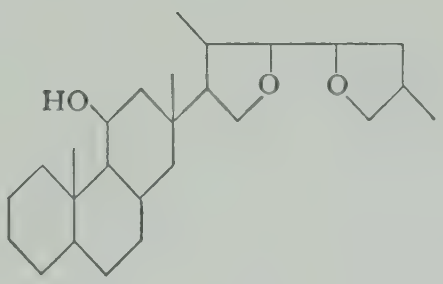
Digitoxigenin



Betulin



Bufotalin



Sarsapogenin

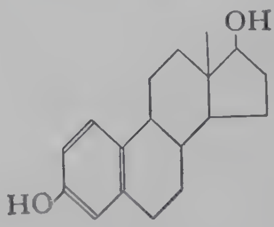
by the Arizona Indians. Saponins are glycosides and the sapogenin or aglyc may be either a cyclised polyterpene, such as betulin, or a steroid with two fu rings in the side-chain, such as sarsapogenin.

At this point the glandular hormones which regulate the sexual cycle in mammals come into the story. A long-continued series of researches for past thirty years has led to the conclusion that the sex cycle is controlled by t hormones or two sets of hormones. The hormone of the ovarian follicles, oestro dominates the first phase of the cycle preceding ovulation (growth of the vagi and of the uterine mucosa); the luteal hormone, progesterone, dominates t second phase, permitting the formation of the placenta (including the chan prior to the implantation of the embryo) and ensuring the continuance pregnancy. Largely through the work of Doisy and of Butenandt (refs. in Fieser the chemical constitution of the follicular hormone was elucidated, and it w shown to belong to the group of sterols, while subsequently the same fate ove took the luteal hormone (Allen & Wintersteiner). The hormones found in t urine differ slightly from those isolated from the endocrine tissue; oestrone a ketone while oestradiol is an alcohol; androsterone is less aromatised in ring than testosterone. Ring I is fully aromatised in all the hormones of the follicul group. Though they have the same effects they differ in potency, those from t urine being not quite so active as those from the tissues. In progesterone t side-chain is not so short as in the others. If ring IV has two hydroxyl grou instead of a keto or one hydroxyl group, as in oestriol (p. 254), occurring human pregnancy urine, the activity is reduced 100 times.

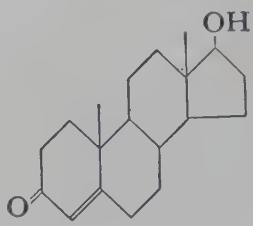
FEMALE

MALE

In tissue

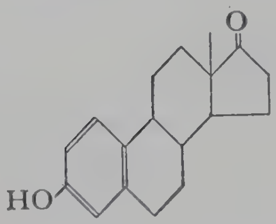


Oestradiol (O)

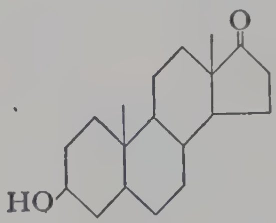


Testosterone

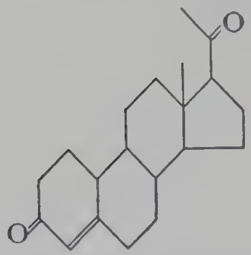
In urine



Oestrone (O, E)



Androsterone



Luteosterone (progesterone)

ie structural relationship between many of these compounds is seen if ing with cholesterol we cut down the side-chain (p. 254). First pregnandiol is uced, an oestrogenically inactive compound isolated from human pregnancy :, then androsterone. If ring I of pregnandiol is aromatised progesterone osterone) results. If pregnandiol is aromatised in ring I, and its side-chain e same time cut down, we obtain testosterone, then on further aromatisation one, and when ring II is also aromatised, equilenin, a weakly active substance ted from horse pregnancy urine. Further aromatisation leads to the carcino- c substances such as methylcholanthrene.

orticosterone (p. 254), the hormone of the adrenal cortex, has since been n to have a structure very similar to that of progesterone (review by Winter- er & Smith).

now appeared that an overlap between the stimulus for cancer production the stimuli of the sexual cycle existed. Aschheim & Hohlweg made the esting discovery* that substances are present in extracts of bituminous rial such as coal and mineral oil, which have considerable oestrogenic ncy. Obviously this might be due to substances with a similar ring structure e naturally occurring sex hormones. In accordance with this line of thought k, Dodds, Hewett & Lawson and Cook, Dodds & Greenwood examined a number of synthetic hydrocarbons (the carcinogenic activities of which known) as to their ability to produce oestrus when injected into castrated nts, or to change the feathers in capons from the characteristic male to the acteristic female colour. It was thus found that high oestrogenic potency possessed by bodies such as 1-keto-1 : 2 : 3 : 4-tetrahydro-phenanthrene. some compounds were both carcinogenic and oestrogenic, such as 3 : 4- pyrene and 5 : 6-cyclopenteno-1 : 2-benzanthracene. Substances may thus arcinogenic or oestrogenic or both (cf. the review of Schmidt-Thomé). This lap is also found on the borderline between the oestrogenic substances and sterols, for neo-ergosterol is oestrogenically active. Oestrogens were later d by Donahue & Jennings in ovaries of invertebrates, and by Skarżyński[1] others in plant tissues, which, though apparently unaffected by oestrone in way, can destroy it and therefore perhaps make it (v. Euler & Zondek). Even gesterone-like substance has been obtained from plant tissues (de Suto-).

rsuing further this chain of relationships, it has been possible to show that varian hormones, interacting with hereditary factors, are responsible for the frequent tumour of rodents, mammary carcinoma. L. Loeb and his borators were able to show that if in mice belonging to strains with a known incidence of cancer the ovaries were extirpated at the age of three months the er incidence fell to zero (Lathrop & L. Loeb; L. Loeb[2]; C. F. Cori; Burrows[1]; oeb, Burns, Suntzer & Moskop). If extirpation was performed at successively periods, the cancer incidence correspondingly rose. Or if the animals were equently given oestrone (Lacassagne[1]) or a synthetic oestrogen such as tri- ylethylene (Robson & Bonser) or stilboestrol (Shimkin & Grady), tumours eloped. Seeing that the normal oestrogens of the body induce periodical th of the mammary gland, it is of great interest that given certain hereditary lispositions, pathological growth may be induced there. Natural or synthetic rogens, however, will also induce uterine fibromata (Lipschütz & Vargas).

nce the first discovery of oestrogenic substances other than the naturally rring hormones and their derivatives, many other substances not possessing

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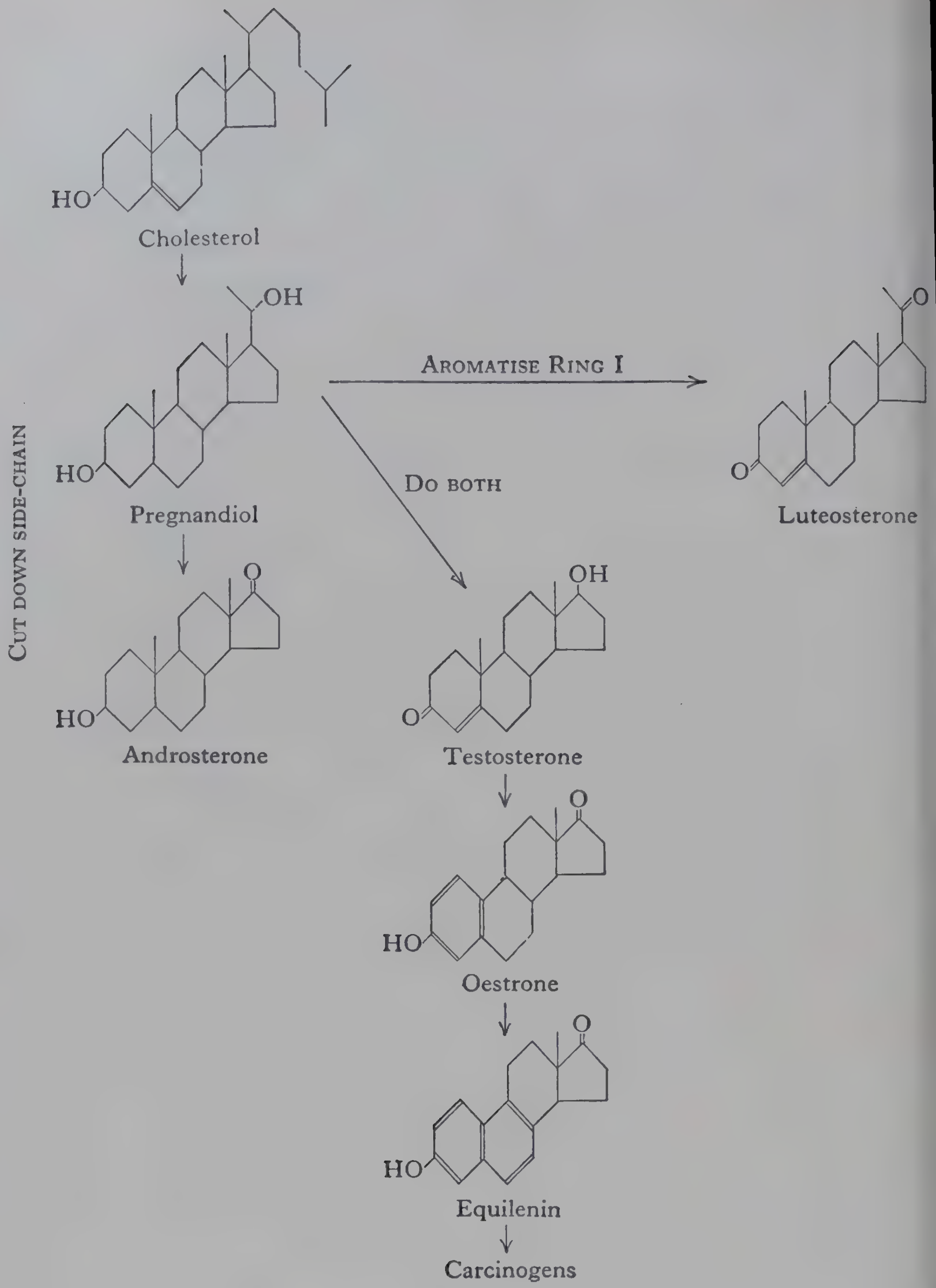
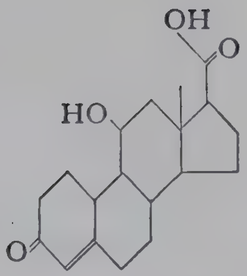
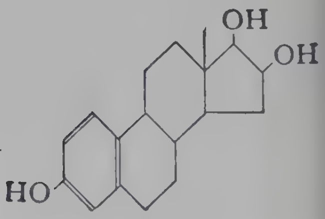
* Later often confirmed, as by Arthus & Provoost.


HO
Cholesterol
OH
HO
Pregnandiol
Aromatise Ring I
O
O
Luteosterone
Do both
Cut down side-chain
O
HO
Androsterone
OH
O
Testosterone
O
HO
Oestrone
O
HO
Equilenin
Carcinogens


Corticosterone


Oestriol

phenanthrene ring system have been found to be active (see p. 256).
wledge of oestrogens has thus developed in the same direction as that of
arcinogens. The simplest of the simple polycyclic systems is the double
of the diphenyls and stilbenes (Dodds, Golberg, Lawson & Robinson;
pbell), such as 4 : 4′-dihydroxy-diphenyl and 4 : 4′-dihydroxydistilbene.
r Dodds, Golberg, Lawson & Robinson reported that the two substances
-dihydroxy-α : β-diethyl-stilbene and 4 : 4′-dihydroxy-γ : δ-diphenyl-β : δ-
diene were several times more potent than oestrone and at least as potent
stradiol. The acenaphthenes are more complicated. Some of them have
found to possess evocator activity and most are polyploidogens (see p. 509).
e search for a monocyclic oestrogen led to the examination of *p*-oxyphenyl-
alcohol, which showed a weak activity. Strong activity on the part of
penylphenol (anol) was later traced to the presence of polymerisation
ucts (Dodds & Lawson), such as di-anethole (Campbell, Dodds & Lawson).
hat has this mass of facts to do with organiser phenomena? The answer
perhaps the problem) is that polycyclic hydrocarbons act on competent
ral ectoderm like the primary evocator. Waddington & Needham (cf. p.
, as soon as the first evidence in favour of the steroid nature of the evocator
was available, tested 1 : 9-dimethylphenanthrene and 9 : 10-dihydroxy-9 : 10-
yl-1 : 2 : 5 : 6-dibenzanthracene (using the di-*n*-butyl derivative), substances
ngly oestrogenic, with positive results (Fig. 92). Later the series was
nded by Waddington[21], and the compounds found to be active as evocators
marked (E) in the accompanying tables of formulae, with the exception
aphthalene, cholesterol, anthracene, squalene, sitosterol and 5 : 6-cyclopen-
-1 : 2-benzanthracene, where the effects were either very small or probably
to contamination with other substances. Chrysene, dihydrocholesterol and
line were tested at the same time with quite negative results. Subsequently
C. Shen obtained many fine inductions with the water-soluble 1 : 2 : 5 : 6-
nzanthracene-endo-α-β-succinate, as already described (p. 182), and the
ute dosage at which this substance was active suggests that the action of the
rocarbons is direct rather than indirect.*

hatever relations there may be between evocators, carcinogenic hydrocarbons,
steroid compounds, there are in the biological literature some curious facts
h would be simply explained if a close chemical relationship were to exist,
which in any case have a significance of their own.

e first is the suggestive series of researches of Witschi[1]. Briefly the facts are
llows: If frog's eggs are kept for various lengths of time before being fertilised
sperm, those which are kept a short time are abnormal in their sex-
rmining mechanism and give an abnormal sex-ratio. Under the best con-
ns nearly 90 % males may be produced, and similar effects are known in
development from the work of Mřsic; Huxley[2], and others. Those eggs which
kept rather longer produce many double monsters, indicating a disturbance
he normal processes of production or liberation of the evocator. Those
ch are kept longer still produce teratomatous proliferations whose malignant
re was tested by the formation of metastases on transplantation to older
ae. Now since we know that the principal sex-hormones belong to the group

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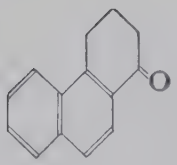
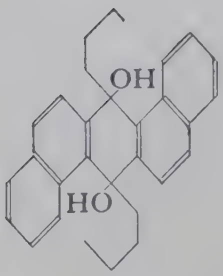
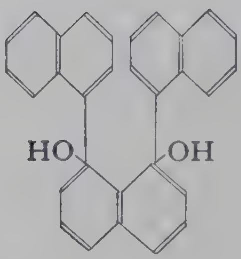
e steroids may, of course, have other, minor, morphogenetic effects, such as the asymmetries of
al fold formation described by Töndury[3], when testosterone, cholestenone, etc. act upon the
g neurula from the outside.



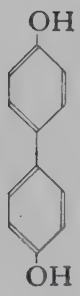
1, Keto-1 : 2 : 3 : 4-tetrahydrophenanthrene (O, E)



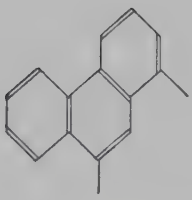
9 : 10-Dihydroxy-9 : 10-dihydro-9 : 10-dialkyl-1 : 2 : 5 : 6-Dibenzanthracene (O, E)



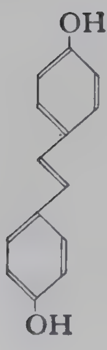
1 : 2-Dihydroxy-1 : 2-di-α-naphthyl-acenaphthene (O, E)



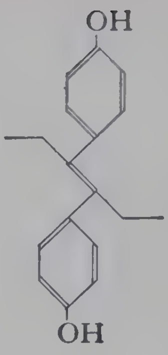
4 : 4′-Dihydroxy-diphenyl (O, E)



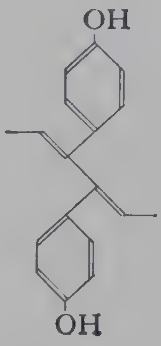
1 : 9-Dimethyl-phenanthrene (O, E)



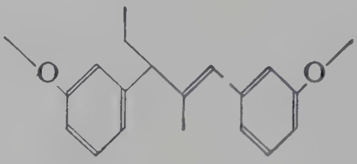
4 : 4′-Dihydroxydistilbene (O)



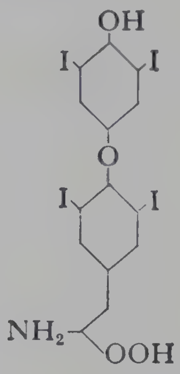
4 : 4′-Dihydroxy-α : β-diethyl stilben
(diethylstilboestrol)



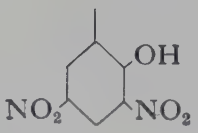
4 : 4′-Dihydroxy-γ : δ-diphenyl-β : δ-hexadiene (O)



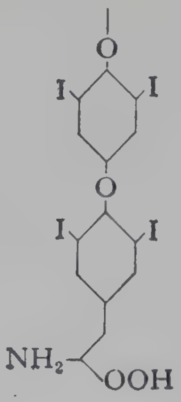
Di-anethole (



Thyroxin



Dinitro-*o*-cresol



o-Acetyl-thyroxin

erols, since we have certain indications that the primary evocator also belongs his group, and since we can be fairly certain that carcinogenic agents have a ted ring-structure, the whole series of effects could be explained on a unitary othesis, namely, that over-ripeness involves a progressive disturbance of oid metabolism.

et us look a little more closely at the malformations which follow medium -ripeness. The simplest were duplicated forms, as shown in Fig. 135, and in e no doubt the neural tubes were joined. Such cases may arise from an tacle in the way of the cell streams during gastrulation, and cannot be certainly rded as the work of accessory nisers. But as the other drawings he figure show, many cases were ained in which secondary embryos w out of the flank or the belly, giving exterior appearance very similar to of induced embryos caused by im- ntation of organiser material or of ve evocator substances. In a later er, Witschi[1] tells us that in a late- ilised embryo there may be seen, at trulation, the formation of multiple stopores. A double breakdown of normal correlating mechanism has refore occurred, first, the inhibitory ctions of the grey crescent individua- n field, which normally bring it about t the blastopore appears at one point l one point only of the egg's surface, failed to act, so that several more ess successful invaginations begin. ondly, the metabolic processes in embryo, which normally lead to the ration of the evocator only in one on, have got out of control and rate it in improper places, such as the lateral and ventral regions. Over- ness acts, therefore, like heat or an organic solvent, in liberating the evocator scriminately. In many of Witschi's cases the secondary neural tubes and chords were quite separated from those of the "host", proving that accessory anisers had been involved.

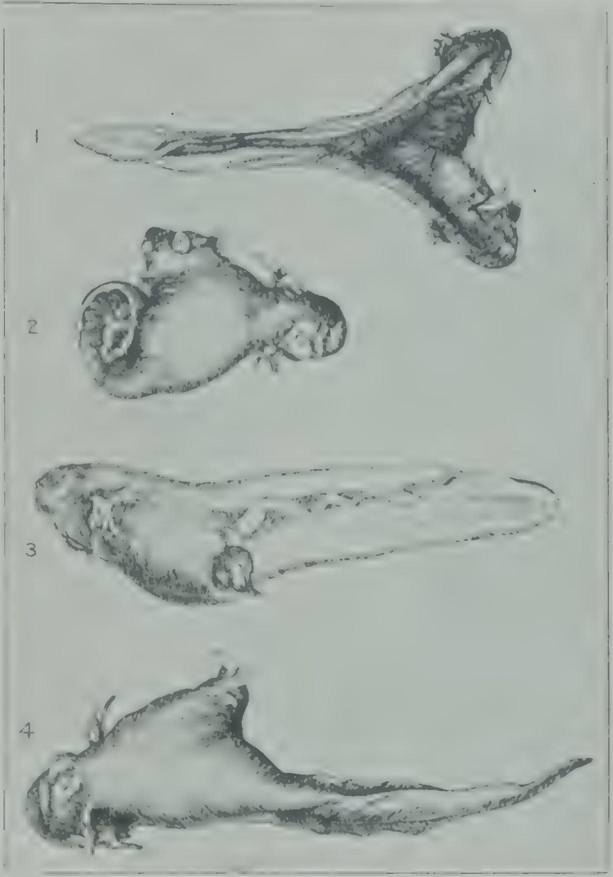


Fig. 135. Malformations caused by fertilisation of over-ripe eggs in the frog.

ut suppose that the over-ripeness has proceeded still farther. According to tschi's description the ectoderm becomes much thickened, consisting of large oidal cells, and "often by a wild growth, gives rise to epitheliomata". The ral tube may be completely missing and in its place there may only be a mass ncompletely neuralised cells. If the brain is formed at all, there may be urbances of second-grade induction, so that a malformed optic cup growing out may cause no lens formation in the skin. Large tumour-like masses ap in the endoderm, and the separation of mesoderm into notochord and som may not even be attempted. Early death ensues.

Could these very interesting tissues be kept alive by transplantation normal animals? Witschi[1] found that they could, and that in many c they grew by infiltrating the reticular system, coming to resemble in appeara the malignant melanosarcoma of man. Then in a just metamorphosed which had carried a strongly growing implant for 22 days, a metastatic nod was found in the connective tissue. The nodule and some other tissues w then transplanted to another frog, which some weeks later developed a la tumour on the peritoneum which was penetrating the bladder and destroyin Further metastases were found in the liver. In this way, the embryonic tis by reason of its clearly acquired malignancy, had lived 92 days instead of 14 or so which it would have survived had it been left in its original isolatio

Three stages would exist, then, in the disturbance of embryonic sterol m bolism by over-ripeness: (1) deviations in the sex-hormones, (2) uncontro liberation of the evocator, (3) production of a carcinogenic agent. Embryo abnormalities are, indeed, of fairly frequent occurrence after late fertilisati (see the paper of Blandau & Young on guinea-pig eggs and Lillie's work annelid development, p. 511 below).

It is unfortunate that no photographs of Witschi's epitheliomata have b published, but it is of interest that during the investigation of evocator act by chemical methods, similar conditions were observed (Needham, Wadding & Needham). In the study of the controls, by implantation of pure neutral f proteins, and animal and plant sterols, it was seen that whereas the implantat of triglycerides produces absolutely no reaction in the ectoderm (Fig. 91 A), implantation of triglycerides mixed with small amounts of sterols, such cholesterol, gave rise to swellings of a characteristic cuboidal epithelium. Th is in such cases a random distribution of cells, and at the edges the cuboidal sh is not lost, so that a saw-like or serrated edge is presented to the exterior. T condition was illustrated in Fig. 91 C. We used to refer to these forms as "ste bumps", and the case illustrated is by no means the most extensive of su thickenings, which sometimes attained a great size. This is certainly proliferat without differentiation. It would be of considerable interest to see whether tis of this sort would show any malignancy when implanted into a larva or an ad frog.

Other investigators have had similar experiences. Tur[2], who has made a stu of the "anidian" blastoderms of sauropsida (see above, p. 223), speaks "neoplasmoid" blastoderms in birds and reptiles. In these cases, the blastode may form an enormously thick mass of ectodermal cells, incapable of nor differentiation, but situated at the centre of an otherwise normal area pelluci The normal ectoderm would be from 15 to 90 μ in thickness; but this material h an average thickness of 180 μ. Tur's discussion[3] attributes a cancerous quality

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* Zimmerman & Rugh could not reproduce Witschi's results, but they worked with differ amphibian species.

: curious formations, which require much further investigation.* One is cted in Fig. 111.

is at any rate sure that the chick embryo and especially the allantois has petence to react to the Rous sarcoma agent. As Rous & Murphy showed, free extract of the sarcoma, applied to the allantoic membrane, causes the luction of sessile nodules as much as a centimetre or more in diameter in the se of a few days. Later, Rothbard & Herman could not get benzpyrene-iced tumours to grow in the chorio-allantoic position, but another, "virus", our, the Stubbs-Furth sarcoma, grew there readily, even when the stimulus applied as cell-free extract.†

similar line of investigation, which has not been followed up much in recent s, was that of workers who implanted embryonic tissues into the tissues of t animals. In some cases cancerous growths, claimed, have been produced by these means. re is, for instance, the long memoir of gololowy in 1918, illustrated with drawings micro-photographs remarkable for their ob-ity. Belogolowy took morulae and blastulae nuran amphibia and implanted them into tissues of adult frogs, or into the body-cavity. many cases, as would be expected, self-rentiation followed, but quite often the *ichball*" came to lie in such a position that n a large number of cuboidal epithelial cells e formed, they penetrated the surrounding ies by infiltration and presented the appear-: of a round-celled sarcoma. Later, work of same kind was carried out by v. Tiesenhausen Skubiszewski on the fowl. Hashed chick ryos implanted intramuscularly or into other ies produced, in a few cases, appearances h this author classified also as "round-celled oma". At this lapse of time, it is perhaps cult to appraise the meaning of such work, but although it was undertaken, loubt, at the instigation of a theory of "cell-rests" which does not much rest us to-day, it may nevertheless have resulted in the discovery of some resting facts, and might with advantage be further pursued.

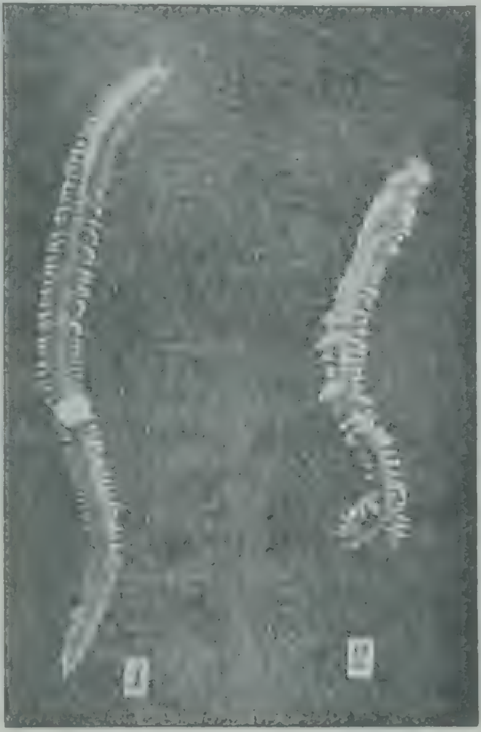

Fig. 136. Spontaneous tumours in the annelid *Nereis*.

ow cases of natural implantation of embryonic material are known, namely, retention within the body of eggs which should have been shed. While king at the marine biological laboratory of Roscoff in France some fifteen

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aestner is sometimes cited as having found neoplastic growth in the early chick embryo. his illustrations show nothing more remarkable than irregular bulges on the neural tube. nours of *late* foetal life are, of course, well known, though rare (Fischer-Wasels; Wells).

was Rous & Murphy's work which led to the use of the chorio-allantoic membrane as a site he culture of viruses; another story altogether. Key references here are the original paper of odruff & Goodpasture, and the monograph of Burnet.

years ago, my wife and I were struck by the frequency with which polychaete wor of the genus *Nereis* carried what seemed to be tumour growths, either of a wh segment, or of the parapodia. This phenomenon was subsequently the subjec a couple of studies by Thomas[1], who showed that it was due to the degenerat of the oocytes which had not been shed as fertilisable eggs. The condition shown in Fig. 136. Later on, the worm often breaks in two at this point, and t normal individuals are formed. The cross-section of Fig. 137 shows the ea stages of a tumour. The degenerating eggs have stimulated the growth o connective tissue which has infiltrated and already quite disorganised the l dorsal muscle. On the ventral surface a proliferation can also be seen, but mu less advanced.*

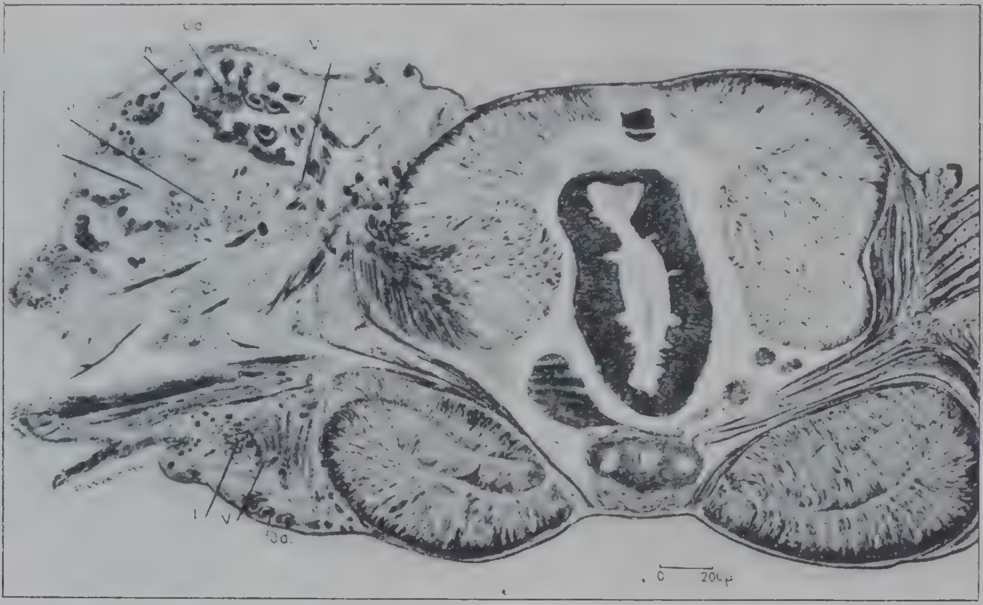

Fig. 137. Cross-section of a tumour in the annelid *Nereis*. *V*, Blood-vessels; *Od*, degenerati eggs; *Rc*, proliferating connective tissue; *P*, parenchyma; *Cl*, free cells; *I*, clump of cells.

An interesting review on cancer in invertebrates was subsequently written b Thomas[2].

Non-embryonic autoplastic transplants have recently been made by A. Fischer who after repeatedly moving a piece of mammary gland around from flank neck, and so on, allowing it to heal in each time, obtained at last more or le malignant carcinomata. He regards metastases as natural autoplastic grafts. A kinds of metabolic derangements might, of course, be expected, in such regraft tissues, not only deviations of steroid metabolism. Goerttler[2], moving in the sam borderland, has implanted pieces of amnion and uterine wall into adult muscl He claims to have observed the development of epithelial cysts and even of ha follicles. He is probably right in thinking that such tissues still contain som curious potencies.

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* Cf. the suggestions of a connection between milk stagnation and mammary cancer in mamma (Fekete & Green; Bagg[5]).

384. The nature of biological specificity

ne facts just discussed have during the last ten years compelled a thorough naul of our ideas of what is meant by biological specificity. As Dodds nsisted in several reviews, there are, beside the normally occurring active tance—the key itself—a number of "skeleton" or even "pass" keys. meaning of this fact is still unknown. It may be that the normal active tance does not act as such at its site of action, but has to be broken down in bolism to something chemically simpler, and that all the other substances h act as well are all capable of being broken down to the identical active ping. Or it may be that in order to act the active substance must combine the form of some complex with a specific protein, and that all the active tances are capable of forming this complex and so presenting their active ping to whatever mechanism it affects. Efforts directed hitherto, however, etermining some common grouping in all the substances which will bring it a given effect, have not so far been attended with success (Friedmann[1]; & Dodds; Cook, Dodds & Warren).

any case, it is clear that there is a considerable overlap between the activities arious substances. The situation may be represented by a diagram such as in Fig. 138. A given biological effect can be produced at will, not by one nical substance, but by any one of a group of more or less related chemical tances. We may therefore represent this by a contour line enclosing a domain. as some of the substances in this domain will bring about other biological ts also, the domains must be drawn overlapping with one another. Such a esentation takes no account of the relative potencies of the various compounds, it would indeed be preferable to show the domains not as overlapping areas, on a three-dimensional diagram as interpenetrating cones with potency as vertical axis. Since the number of compounds seriously studied, however, ill fairly restricted, and since in some cases, such as organiser action itself, uantitative method of dosage has yet been applicable, the two-dimensional entation must for the time being suffice.*

ne central domain of the diagram represents the sterols and bile acids. king it in various directions lie the domains of the alkaloids, the resin acids, aponin aglycones, and the adrenal cortex hormone. Overlapping with it are ains of the anti-rhachitic compounds and the cardiac aglycones; the former use substances (2), (40) and (41) have a weak anti-rhachitic action (Sherman herman), and the latter because substances such as (44) are known with a digitalis-like action. Lower down and to the left lie the domains of the ile" and "female" stimulating substances. The male domain overlaps with female domain because some substances exist, such as (10), (12) and (13), ch have both male, and even both types of female, stimulating properties Deanesly & Parkes; Emmens & Parkes; Korenchevsky & Hall). Substance is neo-ergosterol, which has a relatively weak oestrogenic potency (Cook,

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domain which could also have been included on the diagram is that of the substances inducing ploidy (see p. 509) for both colchicine and the oestrogenic acenaphthene derivatives are igly active in this respect.

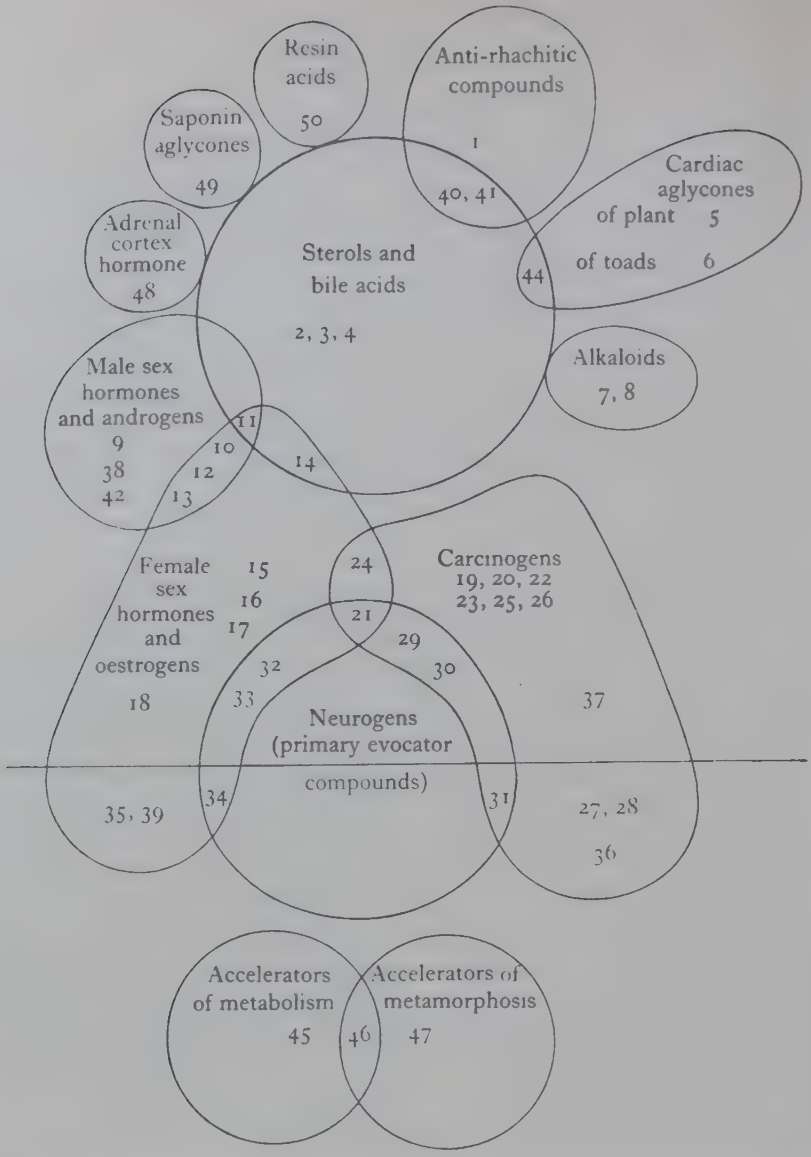
Resin acids
50
Anti-rhachitic compounds
1
Saponin aglycones
49
40, 41
Cardiac aglycones
of plant 5
of toads 6
Adrenal cortex hormone
48
Sterols and bile acids
2, 3, 4
44
Alkaloids
7, 8
Male sex hormones and androgens
9
38
42
11
10
12
13
14
Female sex hormones and oestrogens
15
16
17
18
24
Carcinogens
19, 20, 22
23, 25, 26
21
29
30
32
33
37
Neurogens (primary evocator
compounds)
35, 39
34
31
27, 28
36
Accelerators of metabolism
45
46
Accelerators of metamorphosis
47

Fig. 138. The overlapping domains of biological specificity. Above the horizontal line, conden polycyclic compounds; below it, simple polycyclic and monocyclic compounds.

(1) Calciferol (vitamin D)
(2) β-Cholic acid
(3) Cholesterol
(4) Scymnol
(5) Digitoxigenin
(6) Bufotalin
(7) Morphine
(8) Codeine
(9) Androsterone
(10) Testosterone
(11) Anhydro-oxy-progesterone
(12) Δ^5-Androstenediol
(13) Trans-dehydroandrosterone
(14) Neo-ergosterol
(15) Oestrone
(16) Oestradiol
(17) Progesterone
(18) 1-Keto-1 : 2 : 3 : 4-tetrahydro-phenanthrene
(19) 10-Methyl-1 : 2-benzanthracene (**39**)
(20) 5 : 10-Dimethyl-benzanthracene
(21) 3 : 4-Benzpyrene (**79**)
(22) 5 : 9-Dimethyl-1 : 2-benzanthracene
(23) 3 : 4 : 5 : 6-Dibenzcarbazole (**33**)
(24) 5 : 6-Cyclopenteno-1 : 2-dibenzanthracene (**48**)
(25) 1 : 2 : 5 : 6-Dibenzanthracene (**36**)
(26) 1 : 2 : 5 : 6-Dibenzacridine
(27) Triphenylbenzene
(28) Tetraphenylmethane
(29) 1 : 2 : 5 : 6-Dibenzanthracene-endo-α-β-succinate
(30) Methylcholanthrene (**101**)
(31) Styryl blue
(32) 1 : 9-Dimethyl phenanthrene
(33) 9 : 10-Dihydroxy etc. (see p. 256)
(34) 4 : 4′-Dihydroxy-diphenyl
(35) 4 : 4′-Dihydroxy-distilbene
(36) 2-Amino-5-azo-toluol
(37) 2-Methyl-3 : 4-benzphenanthrene
(38) Androstane-diol
(39) Di-anethole
(40) Irradiation product of 7-dehydrositost
(41) Irradiation product of 22-dehydroe sterol
(42) Δ^4-Androstene-dione
(43) Colchicine
(44) Desoxycholic acid
(45) 4 : 6-Dinitro-*o*-cresol
(46) Thyroxin
(47) Acetyl-thyroxin
(48) Corticosterone
(49) Sarsapogenin
(50) Abietic acid

N.B. Figures in heavy type show relative carcinogenic activity in Cook's units[3].

ds, Hewett & Lawson). The carcinogenic domain does not overlap with the l domain, for up to the present no substance with a typically sterolic ring ture has been shown to possess carcinogenic activity. On the other hand, the oestrogenic and the carcinogenic domains are prolonged downwards to the frontier separating the condensed polycyclic hydrocarbons (above) the simple polycyclic and monocyclic structures (below). There are simple cyclic oestrogens, (35), (39), just as there are simple polycyclic carcinogens, (28), (36).

he compounds which have proved active as "neurogens", i.e. which have ulated the ventral ectoderm of a gastrula to neural differentiation, are shown rming an intermediate domain between the oestrogens and the carcinogens. possible overlaps have been found. Thus one compound, (21), possesses all e forms of activity; two, (29) and (30), are carcinogenic and neurogenic; two, and (33), are oestrogenic and neurogenic, and the overlap is repeated in the of the simple polycyclic compounds just as in the case of the condensed cyclic ones. The question whether these hydrocarbons act upon the ventral derm directly, imitating the action of the natural primary evocator, or in- etly, liberating the evocator present masked in the competent tissue, still ains open, though we have noted evidence (p. 183) that the action is likely e a direct one, since compound (29) acts in such minute dosage.

he overlap between the male and female domains is one of great physiological ortance, though it cannot be dwelt upon here. It is now clear that in the siology of sex-hormones a development has taken place analogous to that ch has occurred in the study of organisers. As the chemical specificity eases, so more weight has to be thrown on to the reactivity, i.e. the com- ence, of the tissues. Zuckerman[2] has pointed out that the testis can produce rogens as well as the ovary, and it is appreciated that the richest source of rone is the urine of the stallion (Zondek). The observation that after ovari- my the implantation of a small piece of testis will restore the uterus to normal ition is now an old one. Both male and female urine and tissues contain both ogens and androgens (Fee, Marrian & Parkes; Womack & Koch). In an esting series of papers Burrows[2]; Burrows & Kennaway and G. van Wagenen studied the effects produced in male mammals by injection of oestrogens, such paration of the pubic bones, testicular hernia, and hypertrophy of the prostate. versely, androgens affect the female mammary gland (G. van Wagenen & ey). Zuckerman[1,2] has shown that these effects can be explained by different tivities on the part of anatomical derivatives of the Mullerian (primary female) Wolffian (primary male) ducts (see p. 311). Thus the hydatids of Morgagni (the igial Fallopian tubes of the male) respond well to the injection of oestrone ckerman & Krohn). Specific competence may therefore be retained by vestigial ans in virtue of their embryonic origin. Conversely, some embryonic tissues m not to have acquired this specific competence. Hain has shown that partial ospadias of the female rat foetus can be caused not only by oestrone but also by ydroxyandrosterone.

he diagram has been amplified by the inclusion, in the region of the simple

polycyclic compounds, of two domains, one representing accelerators of
phibian metamorphosis and one representing accelerators of metabolism.
most classical case of a specific morphogenetic stimulation produced by a
dular hormone is no doubt the effect of thyroxin upon amphibian metamorph
discovered by Gudernatsch[1] and investigated by many workers, such as Hux
After the establishment of the circulation and the development of the thy
gland, thyroxin is poured into the blood at the time of metamorphosis. The
cesses of growth and differentiation which form the hind-limbs are thus activa
the resorption of the tail and gills takes place, and a host of other changes, suc
the perforation of the operculum (in anurans) and the development of the o
ear, follow. All these changes are not due to the specific action of thyro
though they take place much earlier than they would normally if thyroxin is
Many of them involve local specific stimuli of the nature of third- or fourth-g
organisers (see pp. 447 ff.). It is likely, however, that most of them are to s
extent under thyroid control.

Now it is known that in mammals thyroxin considerably raises the b
metabolic rate, but has little or no effect upon the isolated tissue-slice (Dod
Thyroxin also increases the gaseous exchange and probably the heat product
of the tadpole (Huxley[5]; Schwartzbach & Uhlenluth). But the effect of s
compounds as dinitro-*o*-cresol, which in man have a hyperthermising tende
like thyroxin, and which greatly increase both the respiration and the glycol
of tissue-slices, has been tried on metamorphosis by Cutting & Tainter and fou
to be negative. Thyroxin cannot be replaced by them. Conversely, acet
thyroxin, which accelerates amphibian metamorphosis, has no hyperthermis
effect in mammals (Swingle, Helff & Zwemer). We see, therefore, that just as
situation in the steroid realm can be represented by a system of overlapp
domains, so also can that in the realm of accelerators of metabolism and me
morphosis.

In all these questions, the conception of competence must never be forgott
Evidently the tissues of the tadpole are competent to react to thyroxin, sinc
it is fed, metamorphosis will be accelerated, but this is only the case after a cert
developmental stage has passed. Under other names, the conception of co
petence is being widely used at the present time in the experimental pathol
of cancer. Thus amino-azo-toluene produces its tumours not at the point
injection, but only in the liver. The following cases illustrate the conc
in use.

One of the most peculiar of the effects of avitaminosis is that described
Adamstone[1] in the chick embryo if it develops in an egg produced by a
suffering from lack of vitamin E.* About the end of the fourth day of incubat
pathological structures arise in the extra-embryonic blastoderm, forming w
is called a "lethal ring". Intensive cell proliferation in the mesoderm result
the choking of the vitelline blood-vessels, so that the embryo perishes fr
anoxaemia and lack of nutritive materials (see Fig. 139). There is also seri
bleeding into the exo-coelom, usually from the atrium of the heart, at and near

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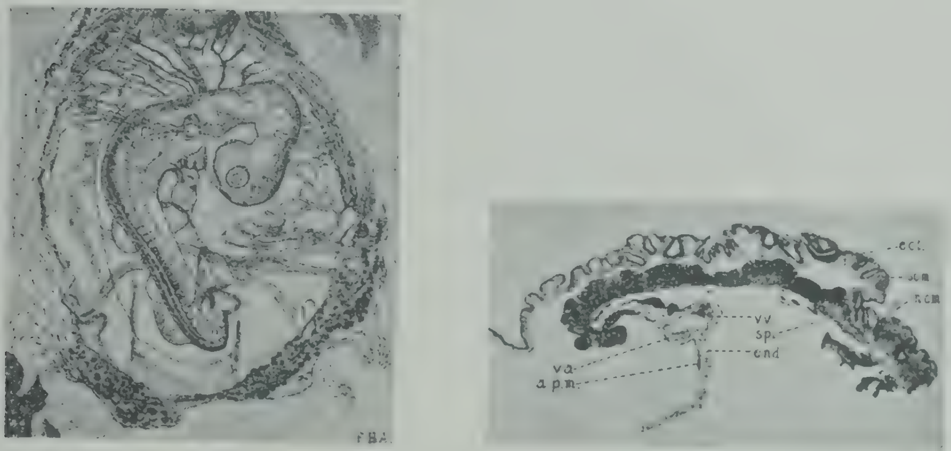
* It is doubtful whether the deficiency here was a pure one.

ture of which peculiar histiocytic mesenchyme cells occur. The general picture mbles that described by Evans, Burr & Althausen for the lethal effect of aminosis E on the developing rat embryo, where wild proliferation of mesoderm ırs, obliterating the placental vessels, together with weakening of the vessel walls extravasation of blood.

'hese relationships might be expressed in the following way. In presence of min E the extra-embryonic blastoderm is not competent to react to the :inogenic stimulus, but lack of the vitamin makes it more sensitive.* Similarly, lt mammalian epithelium is competent to react to carcinogens, but as the 'k of Berenblum has shown, this competence can be abolished by the action mustard gas (β-β-dichlorethyl-sulphide) in subtoxic doses. Such doses, ording to Berenblum, Kendal & Orr, inhibit glycolysis both aerobic and erobic. Chloracetal and its related compounds, too, inhibit glycolysis and ıce competence to react to carcinogens (Crabtree[4]). As Berenblum & Wormall shown by immunological tests, mustard gas effects changes in proteins icient to convert them into proteins foreign to the organism, probably by ıbining with the free amino groups. This gives us a glimpse into the meaning :ompetence, for apart from any action of the substance on enzymes, protein ecules which could otherwise have been oriented into new configurations could ıonger be so (cf. p. 119). It would be of the greatest importance for embryology



A

B

139. "Lethal ring" in the chick embryo in vitamin E deficiency. A. Total mount of 110-hr. embryo showing lethal ring. B. Radial section of blastoderm through the lethal ring (96-hr. embryo). *ect*, Ectoderm; *som*, somatic mesoderm; *spl*, splanchnic mesoderm; *nom*, non-cellular layer of mesoderm; *va*, vitelline artery; *vv*, vitelline vein; *apm*, advanced area of proliferating mesoderm.

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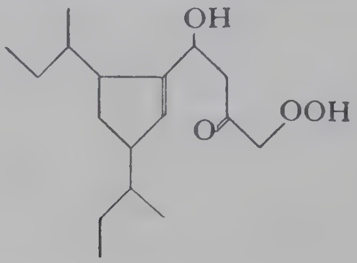
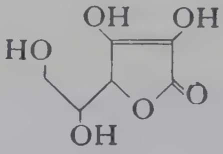
damstone[2] subsequently described lymphoblastoma commonly occurring in young chicks on min E free diets. The formula for vitamin E will be found on p. 266 (and see the review by . Smith). By opening the heterocyclic ring and converting both oxygen atoms into quinonoid ı, a substance active as vitamin K (necessary for blood-coagulation) may be obtained. This he first case in which one vitamin has been transformed into another by a simple nical process. The natural vitamin K is a naphthoquinone derivative with a phytyl side- n (see the review of Weidel), and many naphthoquinones possess some of its activity.

to possess agents capable of enhancing or diminishing the competence of tis
regions for specific differentiations.

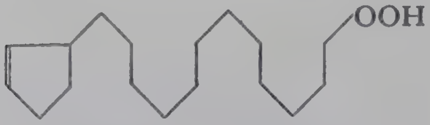
The most natural interpretation of the action of the carcinogens would be
regard them simply as growth-promoting substances acting on single tissues a
forcing them to break the bounds of the animal's individuation field. I
Haddow[3] has shown on the contrary that though the carcinogenic hydrocarbo
do not affect the later growth of a hydrocarbon-induced tumour, they actua
inhibit the growth of transplanted tumours of chemical origin (Haddow
Robinson[1,2]) and of spontaneous tumours (Haddow[2]), and inhibit the growth



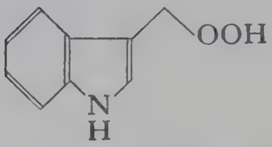
Auxin b (auxenolonic acid)



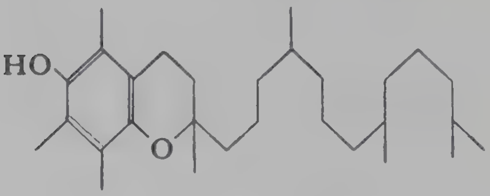
Ascorbic acid (vitamin C)



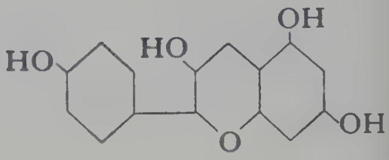
Chaulmoogric acid



3-Indol-acetic acid (hetero-auxin)



dl-α-Tocopherol (vitamin E)



Pelargonidin

the whole animal bearing them (Haddow, Scott & Scott), without interferin
with the animal's metabolic rate or food-consumption (Lees). In general, non
carcinogenic hydrocarbons do not show these effects. In order to account fo
this, Haddow[1] proposed an analogy with the formation of secondary colonies i
bacteria, differing in their fermentative properties from the primary colony
According to this view, carcinogens primarily inhibit growth, until after a whi
some cells, by a kind of mutation, develop resistance to the growth-inhibitin
effect and begin to proliferate uncontrollably. It is doubtful whether th
theory will prove acceptable.* The long latent period (often of months) require

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* A newly discovered fact which shows how cautious we must be in interpreting the Haddo
effect is that the tumour-inhibiting power of the carcinogens has been shown to be very depende
on the nature of the solvent which is used as a vehicle for them (Morelli & Dansi).

ore the carcinogens take effect is certainly mysterious enough. While the w of Haddow[1] would account for the reparation of any inhibition of local, or restoration of general, growth to the normal, it hardly seems to account any precise way for the escape from the organism's individuation field and isequent proliferation *over and above* the normal which the carcinogens iate.*

The only hint we have as to the mechanism of the Haddow effect arises from work of White & White who find that the growth-inhibiting action of hydrobons, not, in their experiments, entirely confined to those with marked cinogenic power, can be fully reversed by the administration of the –SH npounds, cystine and methionine. They suggest that the detoxication of the drocarbons employs sulphur which would otherwise have been available in the dy, e.g. for the sulphydryl stimulation of growth (p. 420). If this were the e, we should expect that small doses would show a growth-accelerating action ile large ones would show a growth-inhibiting one. This is exactly what has en found experimentally by Hearne-Creech in careful work on explants of ouse fibroblasts, and there is confirmatory evidence (Morogami; for yeast, ok, Hart & Joly). That small doses have a different effect from large ones ifter all a common phenomenon in biological reactions, and is shown in cancer elf by the effects of X-rays.

2·385. Homoiogenetic induction and cancer

In the three foregoing sections, we have considered the relation between ganiser phenomena and cancer, first with regard to the escape from the dividuation field, and secondly with regard to the chemical carcinogens. it there is a third point of contact between experimental morphology and ncer research. Since the work of Rous[1] in 1910 it has been known that some nours can be transmitted from animal to animal, not merely by transntation but by the injection of rigorously cell-free extracts. This has led to ery large amount of work (well reviewed by Foulds[1]) and to the hypothesis t a filtrable virus is the causative agent of these tumours, and perhaps of tumours. There has been some difficulty in reconciling this view with the ts which have come to light in the study of the chemical carcinogens. As have seen, there are fair grounds for picturing the production of carcinogenic stances in the body by deviations in the normal metabolism of the steroid oup.

The foundations of the virus theory appear to be two in number, first that icer phenomena exhibit many immunological properties, and secondly that e "virus" multiplies in the host, since after a certain period of tumour growth uch more of the active agent can be obtained from the tumour than what was iginally injected. Now it is interesting that a formal analogy exists between ese types of cancer transmissible by cell-free extracts, and homoiogenetic

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* t is to be noted that, like embryonic inductors, the carcinogens do not seem to affect the process ce it has been set in motion.

induction.* From the work of Mangold & Spemann (see p. 154) we know t
as soon as the neural plate of the amphibian embryo is induced by the organis
influence of the roof of the archenteron, this neural tissue itself acquires
power of inducing a secondary neural plate, when implanted into the blastoc
cavity of another embryo. A whole series of neural plates could therefore
"propagated" in this way, and it is sure that the process could be carried on
implantation not of neural plates but of cell-free extracts of them. There see
thus to be an analogy between the induction of a neural tube, followed by
homoiogenetic induction of successive neural tubes by cell-free extracts of e
other, and the induction of a neoplastic growth by tar or a carcinogen, follow
by the successive propagation of this cancer by cell-free extracts. That chemica
induced cancer *can* be transmitted by cell-free extracts is not perhaps as yet fu
established, but the original experiments of McIntosh have been confirmed a
extended (Parsons; McIntosh & Selbie).

The analogy depends upon the assumption that in the competent ectoderm th
exist stores of the active substance masked probably by combination with a spec
protein. The same may be true of adult animals with regard to an endogeno
carcinogen. Or the synthesis of further supplies may be stimulated autoca
lytically like trypsin from trypsinogen or plant viruses in their hosts (Northro
Bawden & Pirie). The active chemical carcinogenic agents may not be the hydr
carbons themselves, but more or less loose combinations of them with speci
protein. This hypothesis has two advantages. In the first place it would probab
allow for all the immunological and serological phenomena which have led to t
postulation of a cancer "virus". It is not for biochemists or experiment
morphologists to criticise the literature of pathologists, but the study of su
works as the book of Gye & Purdy or the lectures of Rous[2] or of Andrewes h
failed to convince one reader at any rate that it is either necessary or helpful to spe
of the causative agent of cancer as if it were a living organism.† This particul
controversy is no doubt a barren one, since, as Pirie[1] has held, any combinati
of properties may be expected at the borderline between the smallest partic
which we do not hesitate to call living and the largest ones which we do n
hesitate to call non-living. But of the reality of the immunological evidence the
is no doubt, so that specific proteins are clearly involved. Studies such as tho

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* This analogy arose in a conversation with C. H. Waddington and was suggested in a letter
the *British Medical Journal* in 1936. We afterwards found that the parallel between organis
phenomena and filterable cancer-producing substances had been drawn by L. Loeb[4] in 1931 a
the idea that hydrocarbon-protein complexes may be involved had been rather vaguely put forwa
by Pentimalli[4] in 1935. It seems to have puzzled some reviewers. Thus F. E. Lehmann[12] (in t
Ber. u. d. ges. Biol.) thought that the active carcinogen-protein complex was being compared w
an *active* evocator-protein complex. A glance at p. 208 will show that combination w
protein may either inactivate or activate a compound. Foulds[2] (in *Amer. Journ. Cancer*) thoug
that neural evocation could not be compared with carcinogenesis because the latter involv
individuation. A glance at pp. 234, 235 shows that this is the last thing carcinogenesis does!
will be admitted that stimulus to differentiation is a very different thing from stimulus
uncontrolled growth.

† It is an instructive exercise to read through the writings on the virus theory of cancer, su
stituting the words "active agent" or "active extract" for "virus", wherever it occurs. The resu
are illuminating.

ard & Wyckoff, along lines which have been so fruitful with the plant es, will help more than anything else. Perhaps the hypotheses of active and ive hydrocarbon-protein complexes will also be found helpful in cancer rch.

this connection we may remember the sterol-protein complexes with enic properties, reviewed by Marrack, and the compounds of proteins with nogenic hydrocarbons made by Creech & Franks. There are investigators Rous sarcoma virus who claim that part of its active agent may pass through nic solvents (Jobling, Sproul & Stevens; Menke), but this is not as yet rally accepted. Nevertheless Claude[1] has isolated from the Rous sarcoma ex- an extremely active fraction which consists of two parts, a lipoid part account- or 35 % and a protein part accounting for 65 %. The former resembles the hydic phosphatide plasmalogen (see p. 190) and the latter is a ribose-containing oprotein. Most remarkable, however, is the fact that Claude[2] has isolated y similar or identical fraction from 8-day chick embryos, possessing no nogenic activity. The question is, he writes, "whether the main constituents e purified chicken tumour fraction represent inert elements existing also in nal cells; or whether the substance found in normal chick embryo tissue sents a precursor of the tumour principle, which could assume, under in conditions, the self-perpetuating properties of the tumour agent."* Later de[3] succeeded in isolating the same fraction from mammalian embryos, and that it is associated with particles of a particular size (50–200 $\mu\mu$ diam.) cating mitochondria or their fragments. On denaturation by heat or radiant gy, the nucleic acid spectrum appears (Claude & Rothen). These discoveries great significance to the finding of Sturm, Gates & Murphy that the inacti- on spectrum of the tumour agent is that of nucleic acid, and suggest that the k embryo material may be identical with the growth-promoting factor ribed on p. 628; a possibility now under examination by Tennant, Liebow & n.

ne formation of complexes with proteins might perhaps explain the curious ts which many workers have recently been obtaining with the chemical nogens. As detected by ultra-violet absorption spectrography they disappear fowl muscle a few days after injection (Chalmers) and from the circulating d (Peacock[2]). The bulk, says Chalmers, is transformed or undergoes chemical ge. Chalmers & Peacock found that most of the amount injected is eliminated, wrote: "It is possible that the action of the chemical carcinogens is far more d than has generally been assumed, and that essential changes occur in the long before malignant multiplication commences. If the carcinogens act as and over a prolonged time, then the amounts required for carcinogenesis t be very minute, and they must become fixed in some way at the site of ction so as to be protected from the process of elimination." This agrees with the view that a specific hydrocarbon-protein complex has to be

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. the observation of Sharp, Taylor, Finkelstein & Beard that a macromolecular protein fraction similar to that of horse encephalomyelitis may be obtained by centrifugation from normal ay chick embryos.

formed.* The minuteness of the amount probably combining with protein account for the failure of Berenblum & Kendal to find more than traces o hydrocarbons some time after injection when digesting the whole animal potash and examining benzene extracts spectrographically. Moreover, attach to protein might render the substances unstable to caustic potash. Finally, i such experiments it must be remembered that Sannié has shown that the ty spectrum and fluorescence of the hydrocarbons may be quite extinguished i presence of other substances, including proteins.

The objection to the virus theory of cancer lies not so much in that it en with the properties of living matter particles which possess demonstrably some of these properties. An autocatalytic specific protein with a hydrocarb prosthetic group, capable of replicating itself from some precursor substa may be termed a "virus" if this is thought desirable, although some word fewer biological undertones ought surely to be preferred. Plant viruses can be obtained in the paracrystalline and even in the crystalline state. Why sh we not call the primary evocator a virus, since it makes more of itself par with the histological change which it produces? The objection to the virus th of cancer lies rather in the fact that it emphasises primarily the exogenous or of uncontrolled specific proliferation. It has to avoid the difficulty that very cancers are infectious by postulating the existence, perhaps in symbiosis, virus or viruses in most of the cells of the organism.† But on grounds of metho logical simplicity ought we not to prefer the metabolism existing also in all cells of the organism? Rous[2] himself has put the matter well. "The fac obstacles to a parasitic cause for tumours have been whittled down, but theoretical difficulty bulks as large as ever. It takes this form 'all tumours m have the same cause. Certain benign tumours cannot possibly be due to a paras those manifestly referable to developmental anomalies (teratomata) for exam It is impossible to tell of some tumours whether they are benign or malign And so...no malignant growth can have a parasitic cause.'" Rous prefers course the view that all tumours have not the same type of cause. An observe entitled to doubt whether any proof of this negative has yet been given.

The relationships between chemically induced and transmissible tumours now under close examination. The infective Shope papilloma of rabbit skin be transmitted from rabbit to rabbit in cell-free extract, but when maligna occurs it cannot be so transmitted. But if the active principle of the papillo is injected into a rabbit the ears of which have been tarred until the wart st fulminant carcinogenesis occurs and true malignant tumours quickly deve (Rous & Kidd). Parallel experiments have been made with the active princ of the Shope fibroma. Though dibenzanthracene tumours are generally transmissible in cell-free filtrate, they contain an antigen which is capable g rise to antibodies neutralising the active principle of the Rous sarcoma lds[3]). If a dibenzanthracene tumour is growing in a fowl which also bears sarcomata, the active principle of the latter can be isolated from the former lanby[1]) so that a transplant of the former will reproduce itself but a cell-free ct of the former will reproduce the latter. However, all the normal tissues Rous-sarcoma-bearing fowl also contain the active agent (Mellanby[2]) — a ible parallel with the presence of evocators in them but no competence. These ts parallel those of others (Shabad; Neufach & Shabad; Kleinenberg, Neufach abad), who have obtained tumours in mice by injecting benzene extracts of the s or bile of cancerous humans; the tumours were transplantable but not transible in cell-free extract. In their most recent reviews Gye and Cramer[2] lay much hasis on the question of competence, or as they term it, susceptibility. They regard the difference between transmissible and non-transmissible tumours ery mysterious, but is it any more so than the capacity which some enzyme ems possess, and others do not, of coming out into cell-free extracts of tissues?

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* In this connection it is interesting that the effects of the Rous sarcoma agent are greatly enha by the simultaneous injection of the testis spreading factor, now regarded as a mucinase (azoproteins, i.e. serum protein coupled with p-diazobenzenesulphonic acid, which have the s effect), as Claude[4] has shown.

† Is an "indigenous virus" which cannot be cultured by bacteriological methods outside body anything else than just some unknown substance of protein properties found *in* the bo

e may take leave of the cancer problem with a reference to the view which khart-Mummery has gone about to prove, and to which Haddow[1] inclines, ely that tumours are produced by mutations of genes within normal somatic s. In a review, Haldane[2] pointed out that "there is a common and very orderly of change which satisfies Lockhart-Mummery's criterion of mutation, ely the differentiation of cells in the normal course of development. There is evidence that changes of this kind are due to the alteration of a single gene, a great deal to the contrary. Until it is shown that differentiation is due to e mutations, it seems reasonable to regard carcinogenesis as anomalous erentiation rather than mutation." This sentence provides the justification the discussion of cancer phenomena in a book on chemical embryology.

·39. Regional differentiation

2·391. Evocation and individuation

this section we now return to the consideration of normal amphibian lopment. In the introduction we saw (pp. 124 ff.) that it has been found itable to distinguish between two components in embryonic induction: *vocation*, the chemical stimulus to axial neuralisation, and (2) *Individuation*, ield forces which render the ends of this axis asymmetrical by differentiating l end and tail end. What are the facts upon which this distinction is based?

lthough the importance of the prechordal plate (part of the "head-organiser") he morphogenesis of the head had long been appreciated (Kingsbury & lmann; Adelmann[1]), the subject was first approached experimentally in mann's investigation[13] of regional specificity in the organisation centre. aniser material which invaginates first will eventually act as head-organiser itself form part of the wall of the pharynx; organiser material which aginates last will eventually act as tail organiser. Position on the axis implies refore a time difference in the earliest stages, and by taking advantage of this, mann was able to remove organiser material at varying times from the onset astrulation and so have material of varying regional properties available for transplantation. He then implanted "head-organiser" or "tail organiser different antero-posterior levels in host embryos. The four logical possib gave him the following results.

Operation	Result
Head-organiser at head level	Secondary head, with eyes and ear-vesicles
Head-organiser at tail level	Complete secondary embryo, including head, from presumptive trunk and tail ectoderm
Tail-organiser at head level	Complete secondary embryo, including head
Tail-organiser at tail level	Trunk and tail only

These relationships are shown also in the accompanying diagram (Fig. Head-organiser can thus form a head at both head and tail levels, but tail-orga

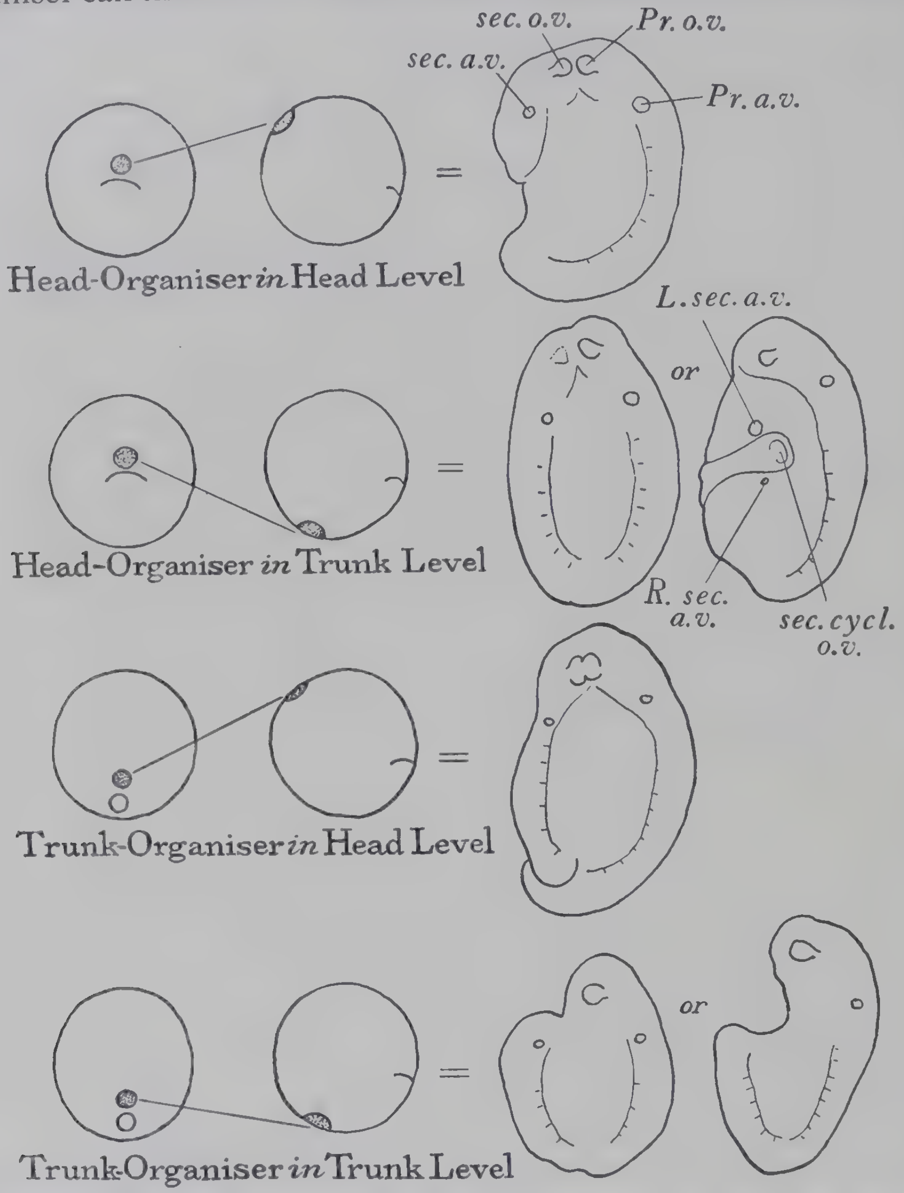
sec. o.v.
Pr. o.v.
sec. a.v.
Pr. a.v.
=
Head-Organiser in Head Level
L. sec. a.v.
or
=
Head-Organiser in Trunk Level
R. sec. a.v.
sec. cycl. o.v.
=
Trunk-Organiser in Head Level
or
=
Trunk-Organiser in Trunk Level

Fig. 140. Spemann's original experiment on the implantation of head- and tail-organise different antero-posterior levels. *Pr.o.v.*, Host eye-cup; *Pr.a.v.*, host ear-vesicle; *sec.* induced eye-cup; *sec.a.v.*, induced ear-vesicle; *L*, left; *R*, right; *cycl.*, cyclopic. (F Huxley & de Beer[2].)

can only form a head at head level. The reaction of the host tissues to tail-organ at head level is to form a head and at tail level to form a tail. These experim pemann's were made using the presumptive mesoderm as such. In later lopmental stages, however, the same regional properties exist, as was shown he notochord by Bautzmann[4] and for homoiogenetic induction by the neural by Mangold[7].

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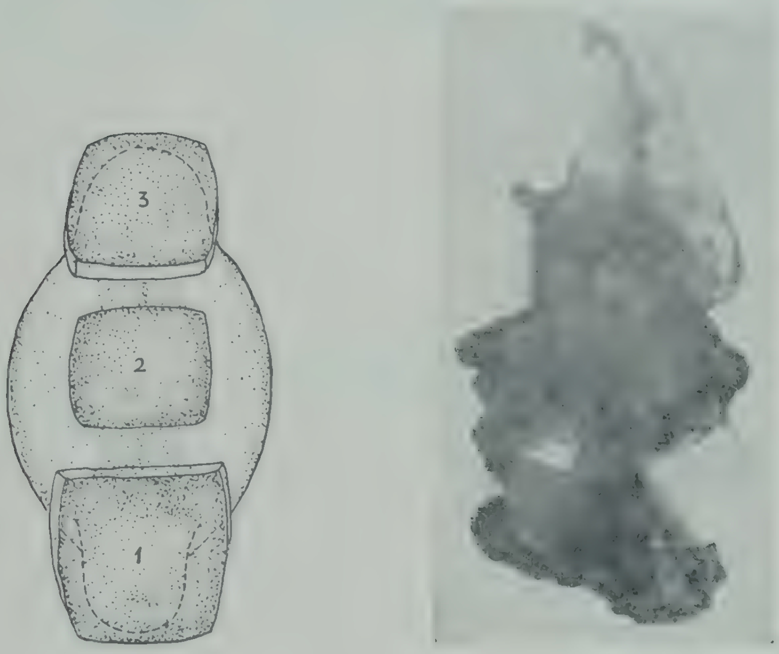
* It is inaccurate to use the word "tail" instead of "trunk" in this discussion, since at the st in question the tail bud has hardly formed, but we do so for the sake of emphasis.

1933, therefore, the paradox had been reached that while on the one hand as clear that a chemical substance or morphogenetic hormone was involved, as also clear that there was some regional differentiation present in the nisation centre. It was this which led to the distinction between evocation individuation. We must now try to analyse the two more closely.

hen Holtfreter's[8] exo-gastrulae (see p. 159) were under intensive study, the ortunity was taken to find out whether the evaginated endo-mesoderm essed regional differentiation. Tests were made in the manner shown in



141. Tests demonstrating the regionally specific inductive properties of the exo-embryo. . Pieces of competent ectoderm laid upon the evaginated endomesoderm. B. Tail induced n such a piece by contact with the posterior end of the exo-embryo.

141. Pieces of ectoderm from various ventral regions of the young gastrula laid upon the evaginated material. After healing on to it they proceeded evelop entirely in accordance with the regional position of the underlying erial; thus a piece placed on the end which had first evaginated produced n, eyes, and ear-vesicles, while a piece placed on the caudal end (that nearest abandoned ectodermal mantle) produced a tail. Antero-posterior regional cificity in induction was thus demonstrated[9] again.

n all experiments where an implantation takes place, as, for example, into blastocoele cavity, there are three factors to be taken into account: (1) the ience of the host axis, (2) the specificity of the implanted material, and any inherent specificity in the reacting material, i.e. the ventral ectoderm. rder to clarify the results which have just been described the third of these factors had, if possible, to be ruled out, and this was done by Holtfreter[5] in transplantations of pieces of ectoderm from various regions of the gastrula various regions of the neurula. Here the competent ectoderm, from whate region originating, showed itself capable of being induced to all kinds structures. The distribution of these is shown in Fig. 142. Thus an open neu plate would be formed in the gill region, or at the level of the ear-vesicle th would arise a piece of brain with an olfactory pit and an eye with a lens. At ot places glands, mesenchyme, cartilage, and muscle developed. Towards the t end head organs were never formed, but notochord, nephridia, dorsal fin, a tails were induced. Fig. 143 shows an implant in the trunk region which w

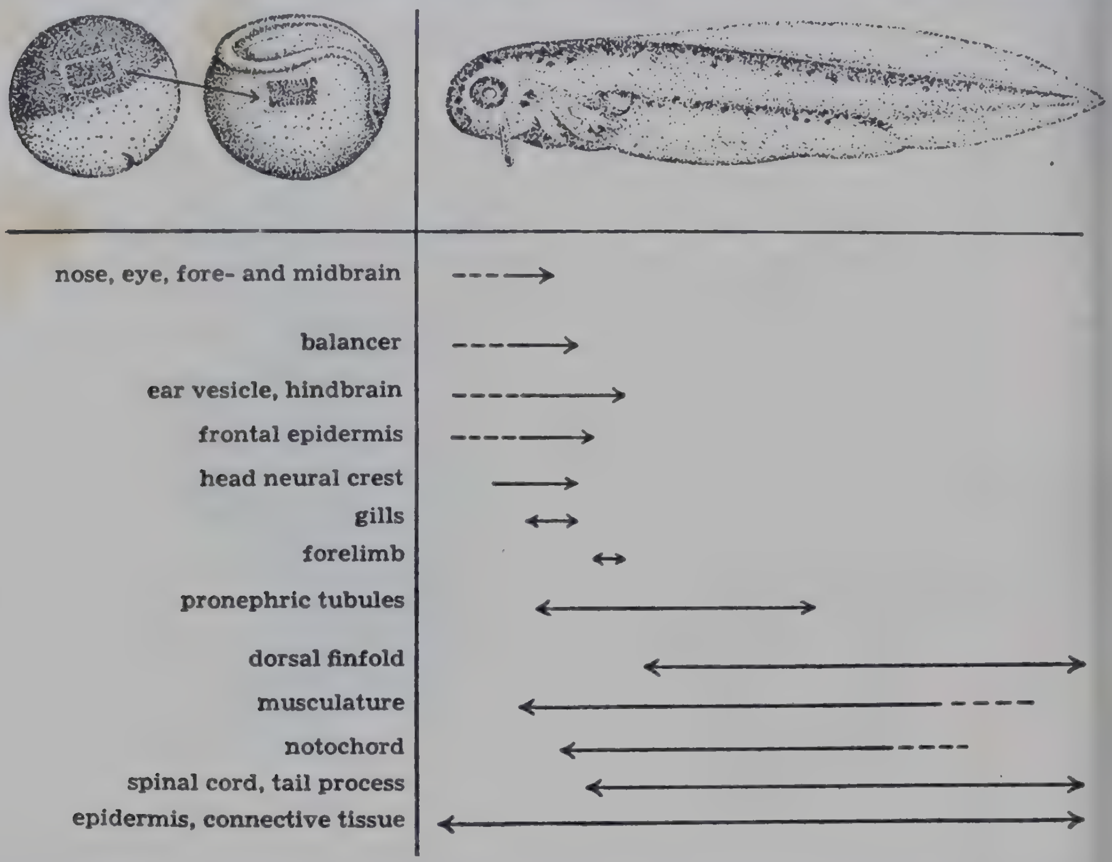
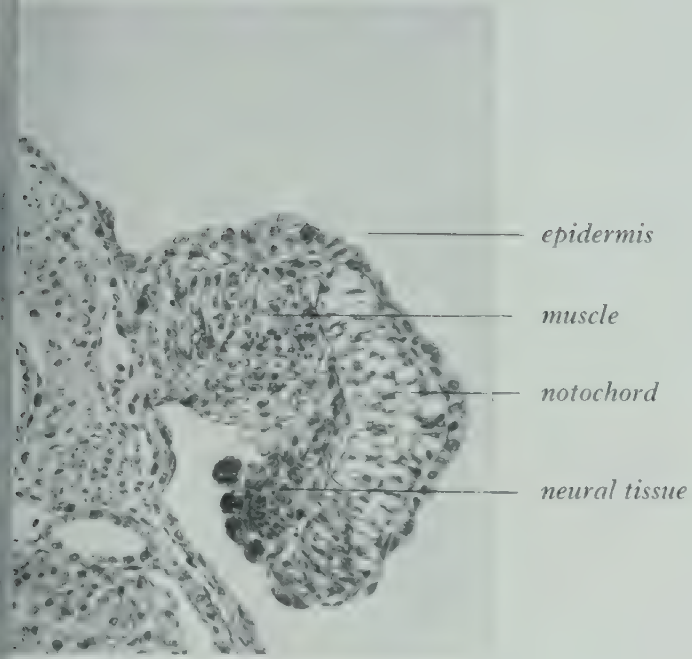

nose, eye, fore- and midbrain
balancer
ear vesicle, hindbrain
frontal epidermis
head neural crest
gills
forelimb
pronephric tubules
dorsal finfold
musculature
notochord
spinal cord, tail process
epidermis, connective tissue

Fig. 142. Diagram showing the results of transplanting presumptive ectoderm or presumpti neural tube tissue from the gastrula to various regions on the flank of the neurula.

induced to form muscle, notochord, and a piece of nervous tissue, all surround by epidermis. The statistical relations between percentage induction of a giv structure and various given regions of the host are also shown below in Fig. 16 to which reference should be made. As there were no differences between t behaviour of various regions of the gastrula, even between presumptive epiderm and presumptive neural plate, this work definitely proved that there is no region specificity in the reacting gastrula ectoderm.

This being the case, we have to consider in transplantation experiments on the mutual influence of host individuation field and whatever specificities t graft brings with it. Normally, as the foregoing experiments have shown, t head region exercises some kind of "dominance", in that all other factors notwit standing, a head tends to be induced in the host's head region, and head-organis


epidermis
muscle
notochord
neural tissue

unk structures, i.e. muscle, notochord,
ous tissue, induced in a piece of com-
toderm transplanted into the trunk
a neurula.

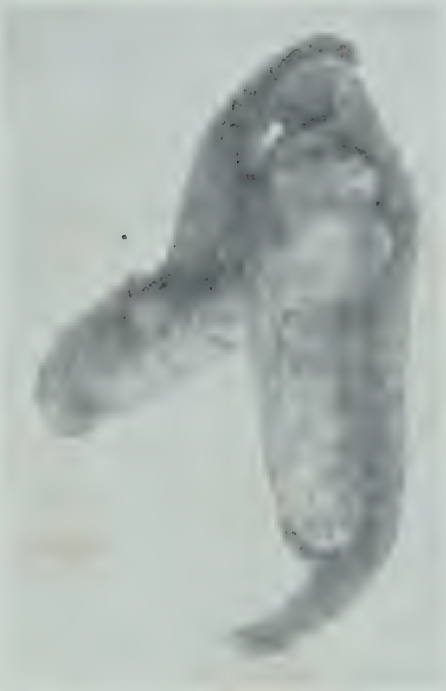
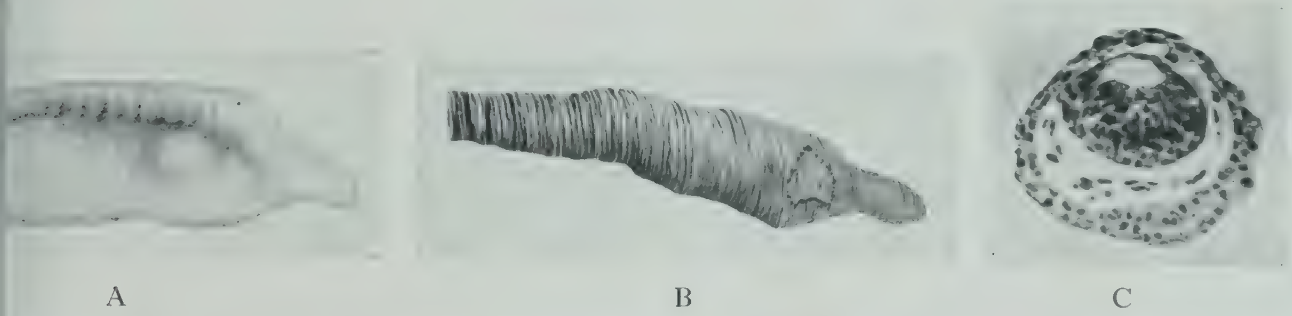


Fig. 144. Secondary embryo oriented almost diametrically opposite to the primary one.



xchange of head- and tail-organisers in the primary axis (Hall's experiment). A. The embryo with
'tail'. B. Reconstruction of the neural tube. C. Cross-section through the anterior region where
and brain should have been.

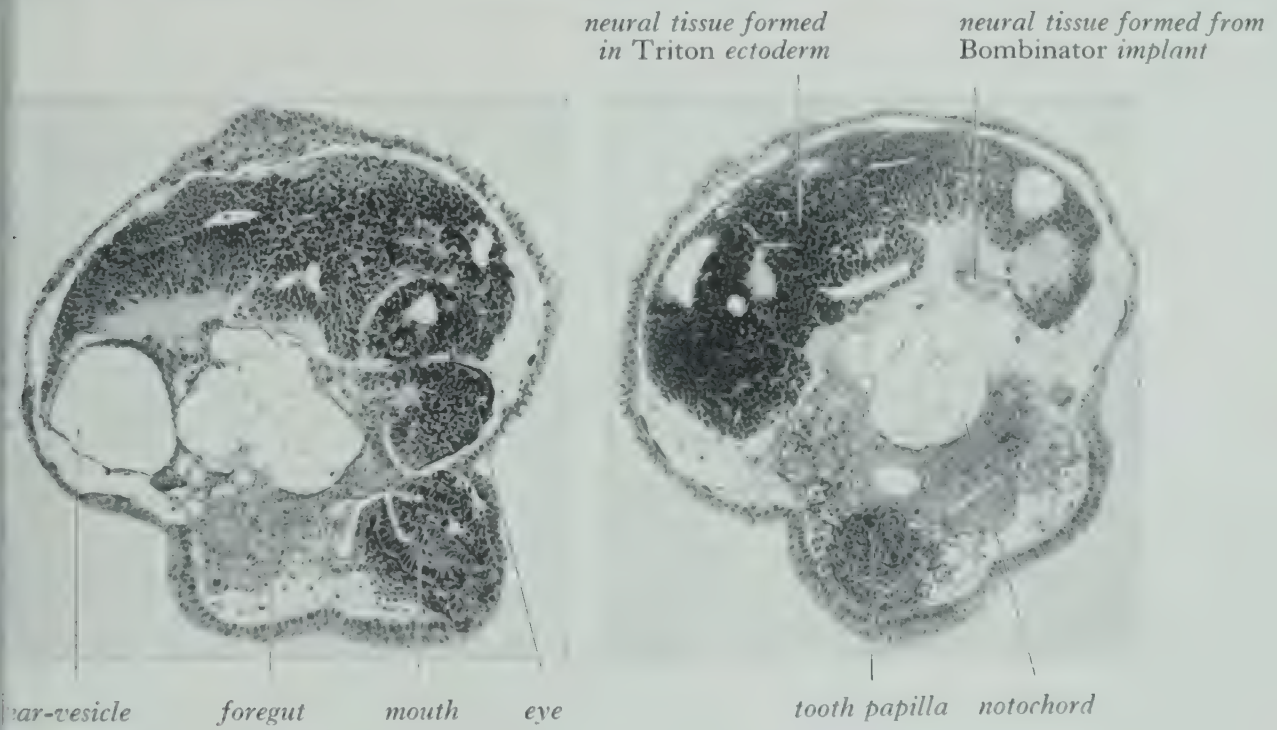

neural tissue formed in Triton *ectoderm*
neural tissue formed from Bombinator *implant*
ar-vesicle
foregut
mouth
eye
tooth papilla
notochord

Fig. 148. Head structures induced in a competent ectodermal ball by implantation of living head-organiser.

ds to induce head even in the host's tail region. But cases are known where this minance breaks down. Thus Fig. 144 shows an induced twin obtained by ltfreter[9] by the implantation of a piece of living dorsal lip. The induced embryo s complete in almost every particular, but oriented in almost exactly the opposite ection to the antero-posterior axis of the host. The host's individuation field y therefore sometimes be incapable of overcoming the field of the implanted ganiser. A similar case had been obtained in the chick on grafting the posterior o-thirds of the primitive streak by Waddington[3], and a number of naturally curring twin embryos described in the monographs of Newman[1] and reste, the papers of Mitrophanov, etc., show more or less opposed embryonic es.

So far we have discussed only the effects produced when two embryonic fields involved, that of the host, and that of the condary embryo induced by the implant. ut if, instead of transplanting early-invaginat- g and late-invaginating organisers to other strulae, we were to exchange them with one other, we should have only the primary nbryonic axis to deal with. This was done in e interesting experiments of E. K. Hall[2], o thus put tail-organiser where the head-ganiser should have been, and head-ganiser where the tail-organiser should have en. The result was that embryos were pro-ced entirely lacking heads, and there was no velopment of brain vesicles or eye-cups; stead, the anterior part of the neural tube s prolonged forwards into an excrescence an "elephant-trunk" (see Fig. 145). Thus -organiser had induced a tail-like neural e at head-level. Correspondingly, no head uctures were induced at tail-level although head-organiser was present there. If emann's experiment[13] indicated a head minance, therefore, that of Hall[2] almost licated a tail dominance. But the two forms dominance are not the same. If normal head-organiser is present in its normal ce in the primary axis it will, as we have seen, usually overcome tail-organiser that level in a secondary axis. If there is no secondary axis, tail-organiser nnot take its place in the primary axis. The regional specificity therefore sides, as so many experiments have shown, in the inducing mesoderm. Head-ganiser in the tail region of a secondary axis will overcome the tail-organiser the primary axis and produce a head. But head-organiser in the tail region of e primary axis, when no secondary axis is present, can only produce tail (see g. 146).

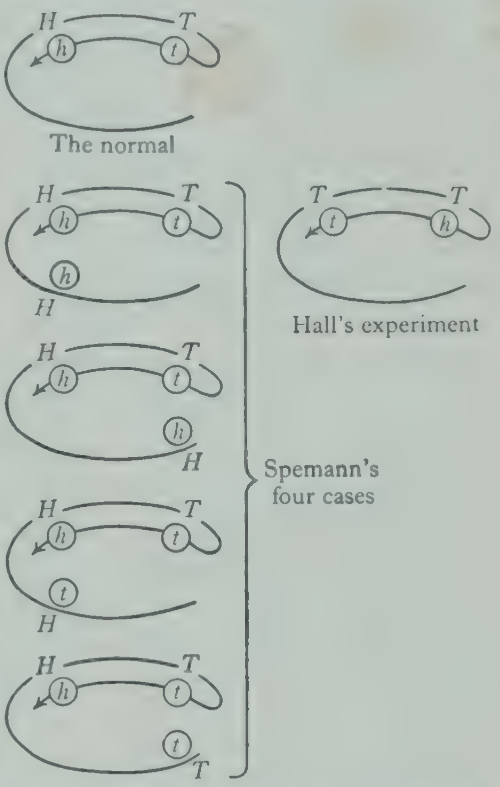


Fig. 146. Comparison of the experiments of Spemann and Hall on regional differentiation. Inductors are indicated by small letters and the result by capitals.

These facts, though fascinating, remain enigmatic. Hall's only explanation was a loss of head-inducing properties on the part of head-organiser when situated in the tail region of the primary axis. But this is only a statement of the problem. Moreover, there are some further possible permutations which have not yet been tried. For example, what would a head-organiser do at tail level if the primary axis was represented there by a head-organiser, potent as regards neural-tube induction but impotent as regards regional specificity? Or what would a head-organiser do at head level if the primary axis was represented there by a tail-organiser forming an "elephant-trunk" excrescence and suppressing brain development? We must await further experimentation.* But in the meantime it is clear that the cautious will avoid giving assent to any theories of "physiological dominance" purporting to explain the original experiments of Spemann.

Of the means whereby head-organiser comes to be different from tail-organiser before invagination has yet begun we know extremely little, save that the part of the presumptive mesoderm nearest the blastopore *is* cephalic and that further away is caudal. Reference has been made already (p. 170) to the remarkable experiment of Töndury[1] in which presumptive neural tissue and presumptive mesoderm were interchanged, so that the former dorsal lip (which should have been organiser) allowed itself to be formed into neural tube, and the former ectoderm (which should have suffered a neural fate) invaginated and actively organised. Liberation of the primary evocator is thus in life absolutely dependent upon the invagination process with its special metabolic characteristics. The experiment was, in a sense, extended to antero-posterior differentiation by Pasteels[13] in the course of his new analysis of "maintained inversion" (Schultze's experiment; p. 218). If an egg which has been held upside down in the one-cell stage, so that the vitelline gradient has been reversed or altered, is severely compressed during gastrulation, it will fail to invaginate and after some hours accessory blastopore lips will form further away from the yolk-mass than the original one. If now the embryo is released from its compression, in favourable cases the newly formed blastopore will have the advantage of the former one and will invaginate forming a well-developed embryo. But the head of this new embryo, with its brain and eye-cups, will have been formed from what would have been, had the original blastopore persisted, "tail-organiser". Thus, wrote Pasteels, "a tail-organiser, maintained artificially in pre-gastrular conditions, may 'ripen' and become a head-organiser".†

According to Lehmann[14], head-organiser and tail-organiser differ in their susceptibility to lithium chloride. The accompanying graph (Fig. 147) shows that section of the antero-posterior axis has its period of maximal susceptibility 6 hr. treatment. It might be thought that this would correspond with the of inrolling through the dorsal lip, but that does not seem to be the case use the anterior head-organiser, for nple, is particularly sensitive some after it has passed through the opore lip.

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* Hall[2] used pieces from younger and older gastrulae; Waddington[27] tried to use pieces from gastrulae of the same age. But the dynamic determination was too strong. Head-organiser insisted on invaginating in the trunk position and tail-organiser would not invaginate at all. Hence there was little regulation; in the former case a rod of pure notochord stuck up through the neural tube. Perhaps a similar effect would explain the *pigtail* lethal in mice of Crew & Auerbach[2].

† Dalcq & Pasteels believe that the difference between head- and tail-organisers is primarily a quantitative one, and they express it by varying values of their C/V product. Certainly each point on the main axis during its formation must have a different value in terms of the vitelline gradient and grey crescent field co-ordinate system. But this explanation will remain verbal until some concrete differences, whether chemical or physical, have been demonstrated between different antero-posterior levels.

the analysis of regional differentiation h help has been forthcoming from the of pieces of ectoderm cultivated in tion away from the individuation of the donor. These round up into suitable for receiving an implant. freter[16] implanted pieces of head-niser and tail-organiser into them, the result that inductions were pro-d entirely according to the specificity he inductor. Examples are shown in . 148 (opp. p. 274) and 149 (opp. 8). In the first of these, head-organiser induced a large piece of brain, an cup, an ear-vesicle, and a mouth-rudiment with tooth-papillae. In the second, organiser has induced muscle, notochord, and tail neural tube.

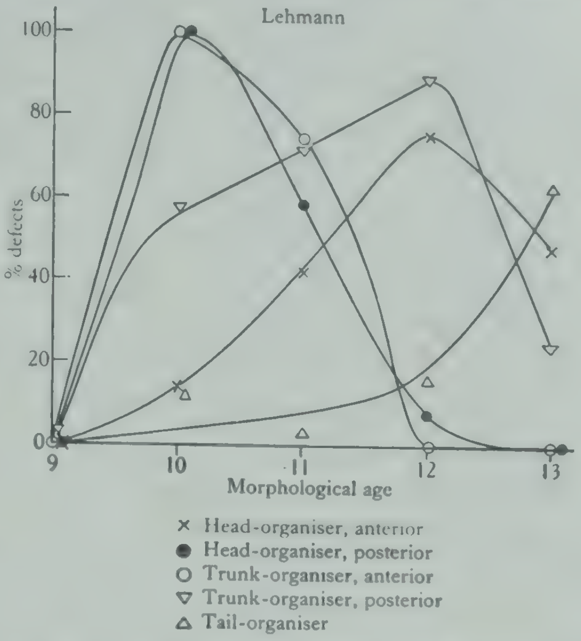

Fig. 147. Specific sensitivities of head- and tail-organisers to lithium.

his regional specificity of the inducing mesoderm was retained for periods o a week if the inductor was cultivated in isolation during that time.

n the other hand, Holtfreter's experiments[16] with pieces of organiser region r heat-coagulation showed equally clearly that the regional specificity was now .* Some of his experiments consisted of implantations of dead organisers the blastocoele cavity of other gastrulae. In these cases the following figures obtained:

	Brains	Noses	Eyes	Ear-vesicles	Lenses	Balancers	Meso-derm
organisers:							
ad-organiser	19	5	5	1	2	9	2
il-organiser	22	3	2	6	0	8	0
ntral ectoderm	12	3	0	4	2	7	1
doderm	7	1	0	2	0	3	2

re was no relation between structure produced and antero-posterior level in host. Thus any dead part of the gastrula would induce almost any organ in living gastrula. It is striking that in these experiments tail-organiser would uce cerebral structures, though, as we have seen from the work of Hall[2], it not do this if it is substituted while living for the normal head-organiser.

o relation was discernible between the manner of killing and the kind of uctures produced by the dead organisers.

ith implantations into isolated ectodermal balls Holtfreter obtained exactly same results. Here the inductions were generally purely neural. An example

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ith[1] thinks that his ultra-violet irradiated dorsal lips had lost their regional specificity, but this ires confirmation.

is shown in Fig. 80; it was produced by implantation of a piece of heat-coagula ventral ectoderm. Such an effect might be regarded as pure evocation, but so kind of specificity might still be carried by the dead inductor. In order to ca the matter further it was desirable to implant into an ectodermal ball isola from the donor's individuation field, not tissue from an embryo, but a pur chemical evocator, and this was done by Heatley, Waddington & Needha Glycogen prepared from mammalian liver (see p. 179), with the ether-solu evocator of unknown constitution adsorbed on it or loosely combined with was used as inductor. The results are shown in Fig. 150. They fairly clos resemble that of Holtfreter just described. Massive neural inductions w produced, but these had not the character either of recognisable parts of t nervous system nor of sections of the normal neural tube. On the other hand, t cells were not oriented entirely at random. They tended to arrange themsel radially surrounding hollow tubular spaces, of which there might be as many six, branching or independent, in one induction. Following on such inducti of neural tissue, the repulsion of overlying ectoderm and the formation of melan phores was observed, doubtless dependent differentiations brought about by t presence of the neural tissue as such.*

Further evidence of the same kind was later obtained by S. C. Shen[2] in rather more elegant way, namely, by immersing competent ectodermal balls very dilute solutions of the water-soluble hydrocarbon derivative, 1:2:5: dibenzanthracene-α-β-endo-succinate. Although in some cases fairly typic cross-sections were seen (as in Fig. 151) they were due to balls, not tubes. further differentiation was found. These experiments, in which a patternle inductor acts on patternless ectoderm, are of much interest, as ruling out mechanical effects due to the solid implant.

The question asked on p. 125 was thus answered. Pure neural evocation do not involve the formation of a regionally undifferentiated neural tube with norm cross-section; it simply means histological differentiation together with a tenden to radial arrangement around cavities.

2·392. Individuation as the organised action of eidogens

How we are to interpret the remaining factor of induction, individuation, one of the most difficult problems confronting embryology to-day. While it convenient for purposes of discussion to speak of the individuation field, we mu be careful that this concept is not used in such a way as to sterilise furth research. The possibility is by no means excluded that other chemical su stances may be at work in the formation of the normal neural axis besides t primary evocator. The existence of second-grade inductor substances is, of cours beyond doubt. Thus, as we shall see in Section 2·395, Lopaschov[5] has report that if the eye-cups are removed from several neurulae, coagulated by heat an implanted into isolated ectodermal balls, they may induce new well-shaped ey

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* Spongy polytubular masses of neural tissue rather reminiscent of these may also be produc if the determined neural tube is removed after its closure and cultivated *in vitro* without the unde lying notochord. This type of anomalous differentiation was described by Monroy[1] (see also p. 28

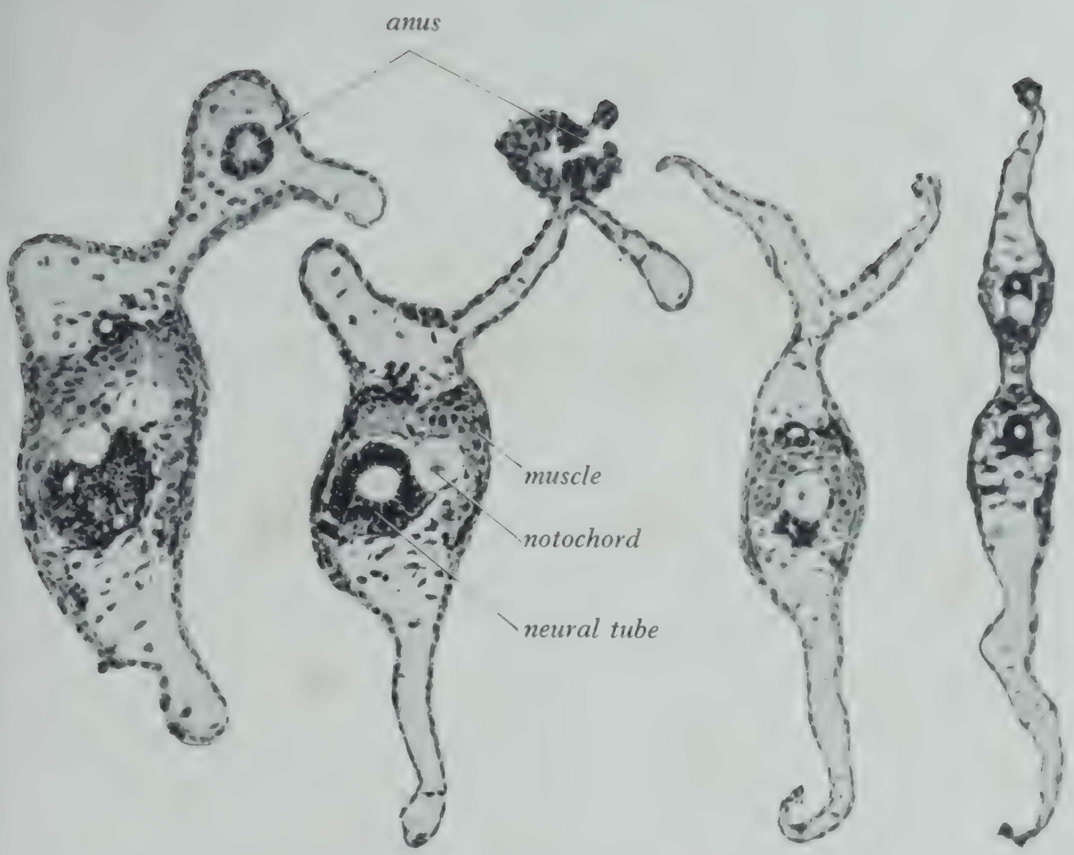

Fig. 149. Tail structures induced in a competent ectodermal ball by implantation of living tail-organiser.

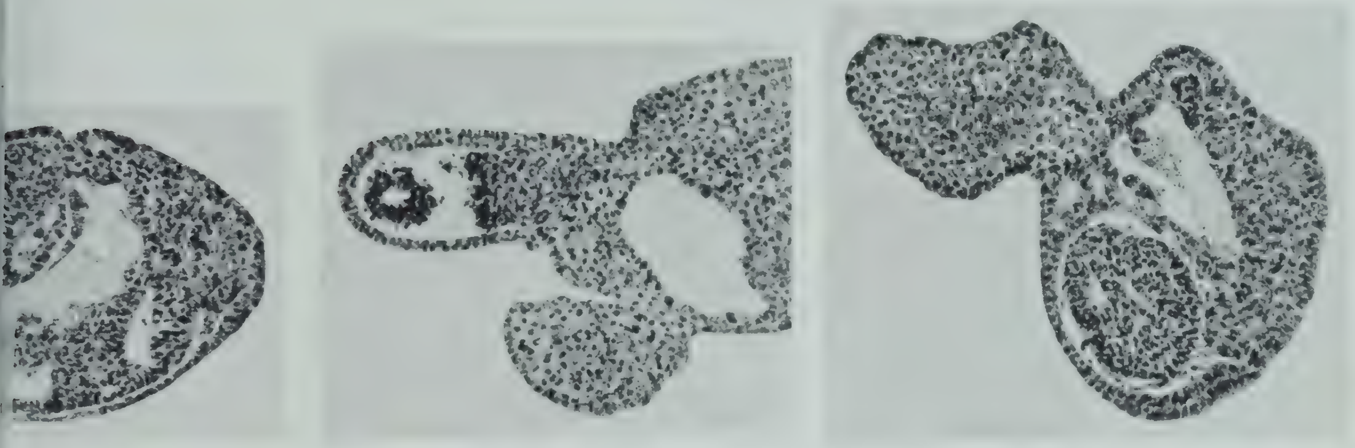

Fig. 150. Neural inductions produced in competent ectodermal balls by implantation of the glycogen-evocator complex.

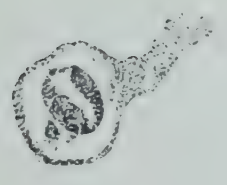

Fig. 151. Neural induction produced in competent ectodermal ball by immersion in very dilute 1 : 2 : 5 : 6-dibenzanthracene-α-β-endo-succinate solution.

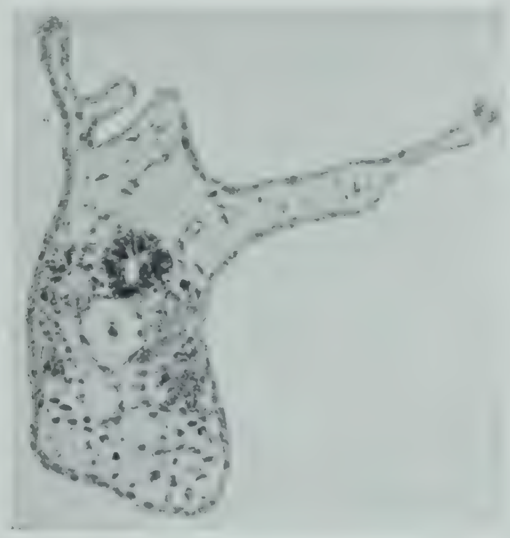

Fig. 152. Induction produced by implantation of adult tissue into competent ectodermal ball.

.* This must be regarded as a homoiogenetic induction by a second-grade
ctor. But we may also find it worth while to distinguish between substances
h stimulate tissues to form an organ (inductors) and substances which modify
arious ways the shape of organs so induced.

such substances exist, it is at any rate clear that the position in which they
normally liberated is so exactly controlled by the individuation field of the
cing tissues that the regional correspondence of inducer and induced is
red. Waddington[20] has discussed the possible existence of these substances
idogens in a short review.† The most important thing which head-organiser
as opposed to tail-organiser is to modify the cross-section of the neural
to form brain rather than spinal cord. Inductors and eidogens‡ would thus
he mechanism by which the individuation field of the inducer controls that of
induced.

n the analogy with evocators of all grades (see p. 302) eidogens might
xpected to be present in adult tissues, and the way was therefore open for
H. Chuang[1] to make the interesting experiment of implanting pieces of adult
ns (which as we have seen have inducing power; p. 172) into isolated ecto-
nal balls. Using fresh adult newt liver and mouse kidney, he found that the
s of induction produced by these implants were significantly different. Not
ely evocated neural tissue appeared, but neural tubes, eye-cups, elongated
chords, nasal grooves, etc. etc. An example is given in Fig. 152.

Here a completely heterogeneous inductor," said Chuang[1], "not itself taking
in the induction, brings into being typically formed, complex, and highly
viduated structures" in the ectodermal ball. The statistics were as follows:

	% Inductions							
	Brains	Eyes	Noses	Ear-vesicles	Balancers	Tail	Muscle	Noto-chord
t liver	85·2	7·4	16·5	74·1	22·2	48·1	59·2	51·8
se kidney	100·0	23·7	44·3	23·7	11·3	0	0	0

s contrary to expectation, liver tended to give rise to more caudal organs and
ey to more cephalic ones. When a similar statistical analysis was made of the
lts of implanting liver and kidney into the blastocoele cavity of intact embryos,
effects of the two organs were almost identical. The individuation field of

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paschov found that no definite eyes were induced if less than a certain quantity of dead
rial was employed, but it does not seem possible to attribute the lack of definite organs in
xperiments of Heatley, Waddington & Needham to a quantitative lack of evocator, since
nasses of neural material obtained often filled up the greater part of the explant.

he word "modulator" was suggested by Waddington[20] in ignorance of the fact that Bloom
Weiss[14] had proposed the use of the term "modulation" to describe all reversible physiological
nges in histological structure, such as those occurring at the final stages of differentiation.
se may be far-reaching enough, as in the spurious de-differentiation shown by some tissue
ures. I shall therefore use the word "eidogens" here to mean substances (possibly allied
ns of one evocator) which differentiate further the product of an evocator stimulus in the
e of the induction field in question, or which modify the shape and form of organs already
ced (from εἶδος, form).

such eidogens are present it is strange that in Hall's experiment they fail completely to act
n transposed.

the host was capable, therefore, of completely masking the specificity which been revealed in the previous experiments.

It is also important that the effectiveness of these adult tissues depends systematic way on the length of time for which they have been boiled. While m of the inductions fall off statistically, eye and nose inductions actually increase to 15 min. boiling and then fall off, as if an inductor was first liberated from active combination (see Fig. 153). Notochord and pronephros inductors, mo over, are more sensitive to heat than tail and muscle inductors, and these in t much more so than the inductors of brain and ear. Everything points to t existence of a series of compounds or complexes differing in their reactions high temperature (cf. pp. 172, 173).

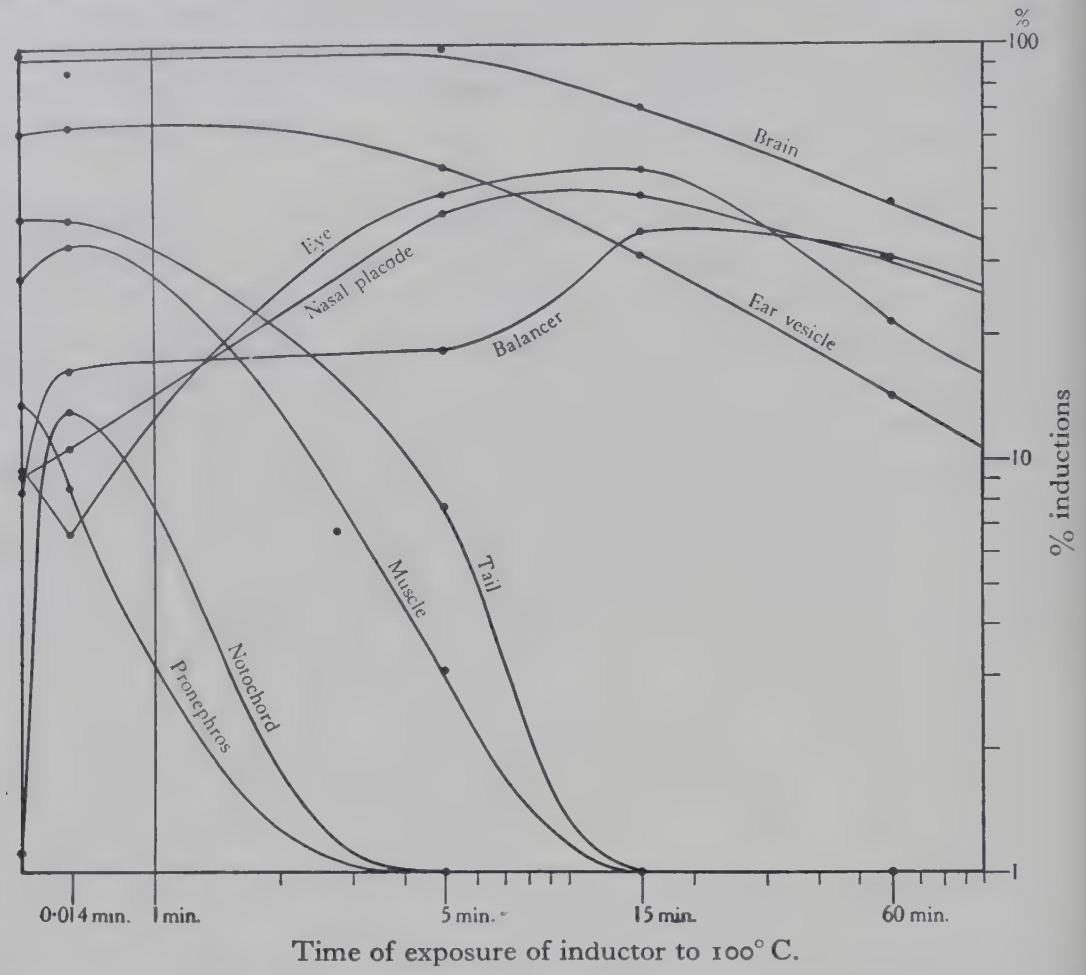


Fig. 153. Relative sensitivity of the various inductors to prolonged exposure to 100° C.

Chuang's work was independently continued by Toivonen[2], who made o implantations of alcohol-coagulated tissues into the blastocoele. It was fou nevertheless that liver (whether from fish (*Perca*), snake (*Vipera*), jay (*Perisore* or guinea-pig (*Cavia*)) always had a tendency to induce eyes, noses, forebrai midbrains, and balancers, while kidney induced hindbrains, ear-vesicles, gil notochord, somites and tails, and heart muscle induced all organs. Guinea- thymus proved to be a powerful and specific inductor of solitary lenses (p. 299), with little other activity.

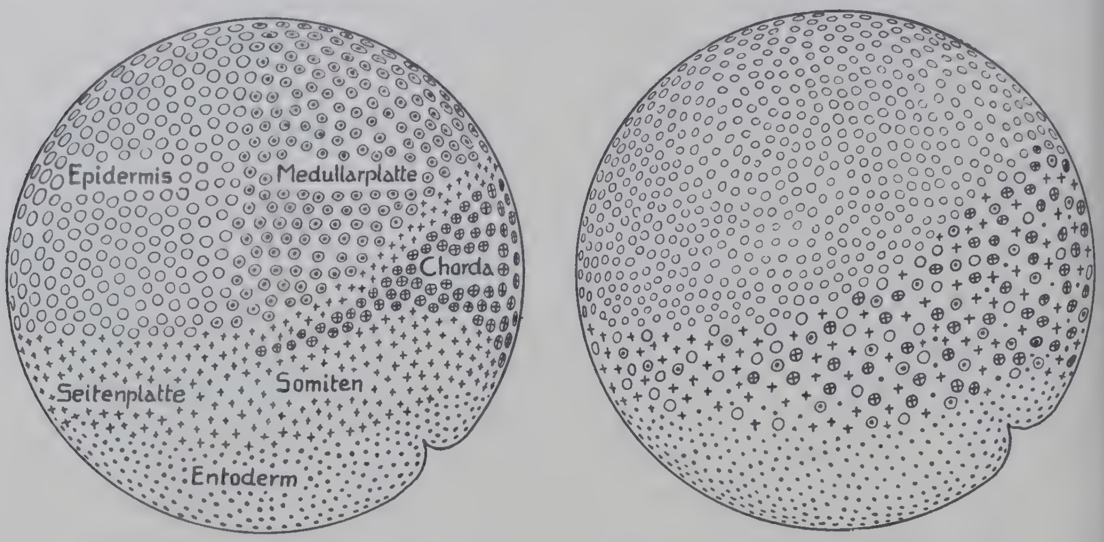
'here is no doubt that the actual mechanism of the individuation field is a le interplay of evocators and competences. At first, gastrula ectoderm is i-competent, it may even accept a mesodermal or endodermal fate. Later it mpetent for neural-tube formation; later still, only for the formation of such ctures as lens, balancer, or gills. Waddington[12] has drawn attention to the ation in the flank of the embryo (cf. p. 153). The just-formed side-plate meso- n from the gastrula in the middle yolk-plug stage can induce the formation of condary neural plate when implanted into the blastocoele cavity of a young rula. It therefore possesses inductive power. Conversely, the dorsal lip of oung gastrula, substituted for the side-plate mesoderm just mentioned, cates a neural plate on the flank of the embryo. Therefore the flank ectoderm mpetent. Yet in normal development a neural plate does not appear on both s of the embryo as well as on its dorsal surface. This remarkable paradoxical lt may be explained by assuming a quantitative diminution in inductive er and in competence, so that the side-plate mesoderm evocates less strongly the dorsal lip, and the lateral ectoderm reacts less readily than the young rula ectoderm. This would be part of the mechanism of the individuation l, but it is quite possible that there may exist an active suppression of the ictive power of the side-plate mesoderm by the rest of the mesoderm.

'he loss of competence in ectoderm does not seem, however, to be dependent n its continuous existence in the organism's individuation field. This loss ceeds just as well when the ectoderm is isolated and cultivated apart in neutral ie medium. In connection with the experiments on the situation in the lateral y-wall which have just been described, Waddington[12] cultivated young gastrula derm *in vitro* until the controls had reached the open neural plate stage, then lanted archenteron roof into it, and obtained some neural inductions. The derm seemed, therefore, to retain its competence somewhat longer in isola- than *in vivo*. But in Holtfreter's exo-gastrulation experiments[8], all neural petence was eventually lost by the abandoned ectodermal mantle. Lopaschov[1], allowing isolated gastrula ectoderm to age *in vitro*, transplanted it on to ous situations of a neurula, obtaining balancers and no neural inductions. experiments were few, however, and it was left for Holtfreter[18], in a work cially devoted to this question, to give a comprehensive timetable of the loss irious competences in isolated ectoderm. This table was as follows:

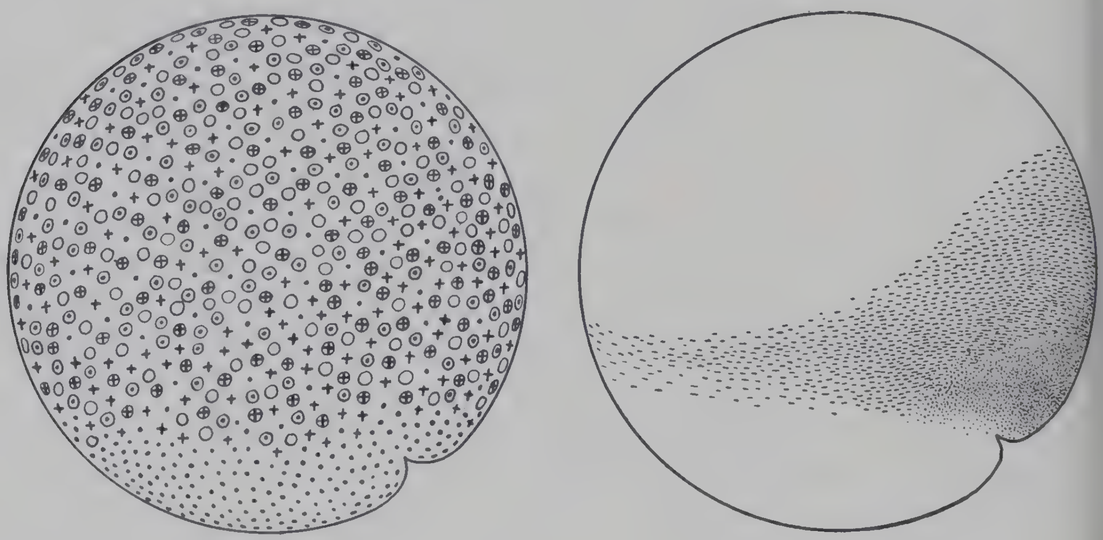
	Inductions following transplantation to neurula situations						
's from ation	Brain	Neuroid vesicle	Neural cells	Ear-vesicle	Mesenchyme	Balancer	Muscle
0	+	+	+	+	+	+	+
15	−	+	+	+	+	+	−
24	−	−	+	−	+	+	−
46	−	−	+	−	+	−	−
4–96	−	−	−	−	−	−	−

is after 15 hr. isolation competence to form brain masses was lost, but com- nce to form chaotic groups of neural cells was retained till the 46th hr. ipetence to form an ear-vesicle was retained longer than that to form muscle. general this agrees with what takes place in the intact embryo; it seems therefore that the loss of competence is not due to its active suppression by organism's individuation field.

But that inhibitory effects do occur in normal development as part of individuation field seems very likely. For instance, Balinsky[7] has described appearance of supernumerary balancers, even up to eleven on one embryo, af removal of most of the endoderm. This he interprets as being due to the remo



(*a*) Prospective significance (*b*) Self-differentiation



(*c*) Prospective potency (*d*) Inductive power

Fig. 154. Holtfreter's maps of the properties of regions in the early amphibian gastrula.

of inhibitory influences. There is also the well-known fact that any piece anuran ectoderm when isolated tends to produce one or more suckers. How these inhibitory effects are due to mechanical relationships which operatio disturb, cannot as yet be decided.

One genuine inhibitory effect, at any rate, is the influence exerted by prechordal plate (the anterior end of the notochord) on the presumptive e districts lying above it. After a great deal of confusion about the origin cyclopia, Adelmann[3] succeeded in showing that normally the two eye-distri overlap and that the development of this intermediate material into eye ibited by the prechordal plate. If this is interfered with, whether mechanically y the action of lithium (see p. 226), cyclopia results. Adelmann regards this ibitory effect as analogous to the contiguity effects described below.

nother way of examining the action of the individuation field is the study of regulations which the isolated organiser region is capable of effecting. In experiments of Holtfreter[19] (p. 158) we have already seen that very many ctures can be produced by the isolated organiser region. Holtfreter[20] brought ther the facts in the following way (Fig. 154), using the classical terminology. prospective significance of the parts (*a*) follows naturally the Vogtian fate-map p. 145). The prospective potency of the parts (*c*) is very loose; apart from the -endoderm, any piece of the early gastrula can turn into any later derivative Bruns; Töndury[1]). The region where the primary evocator will be liberated is, of course, the dorsal blastopore lip. But the self-differentiation map (*b*) ws that while yolk-endoderm will always remain endoderm, and while both sumptive epidermis and presumptive neural plate will always remain ectoderm, organiser region has the power of producing notochord, neural plate, ectoderm somites. Lopaschov[2] has now shown that exactly what will be produced by ated dorsal lip regions depends upon their size.

f one dorsal lip region from one embryo is isolated, only notochord and muscle ments usually appear. If, however, several pieces of such material, derived n several gastrulae, are placed together, they fuse and later produce a large nber of tissues, regulating, indeed, in the direction of a whole embryo. The owing table shows that a direct relation exists between the size of the isolated s at the outset and the degree of individuated complexity afterwards produced:

o. of sal lips ised ether	Noto-chord and muscle	Epidermis	Pigment cells	Neural tissue	Brain	Eye	Lens	Ear-vesicle
–4	+	–	–	–	–	–	–	–
–5	+	+	+	–	–	–	–	–
;	+	+	+	+	–	–	–	–
-10	+	+	+	+	+	+	+	+

accompanying photographs (opp. p. 290) show a fused "organism" made ven dorsal lips. In the first (Fig. 155 A) two brains, a notochord and an earle are seen; in the second, further along the organism, there are three chords, traces of neural tissue, a piece of gill and an eye with a lens . 155 B).

he Russian school of experimental embryology has done good service in ving attention to the importance of the quantitative aspect. Thus Dragomirov[3] nd that pieces of pigment-epithelium of the eye, if sufficiently large, would, n isolated, regulate into complete eye-cups, but if below a certain size would ely form bladders of pigment epithelium. Similar facts have been noted in case of regenerating limbs.

)ne of the most remarkable properties of the individuation field is its capacity of nerating bilateral symmetry when this is destroyed. Thus it was often found e earlier work (and confirmed in special *ad hoc* investigations by Ekman[2] and Mayer[1]) that the lateral half of a dorsal lip, placed in the blastocoele cavity, ices a bilaterally symmetrical secondary embryo with fully paired organs.

2·393. Contiguity and affinity effects

Included in the conception of the individuation field must be all those eidoge influences of one organ or structure upon another which have been described un the head of *Nachbarschaft*; "neighbourhood" or contiguity effects. Holtfrete found, from his numerous explantation experiments in which presumptive tiss were combined in all sorts of permutations, that the normal configuration of t neural tube, for example (*typische Gestalt*), was only reached under cert conditions. Consider Fig. 156. If neural tissue differentiates in isolation the ce round up into a spherical or rod-like structure, with the nuclei at the exterior (If they differentiate within a mass of mesoderm a hollow ball or tube is forme with the nuclei usually at the interior (*b*). If the neural induction is performed muscle, the tube formed will tend to show an excentric lumen (*d*). If there insufficient mesoderm the tube will not close (*c*).* Only if the induction is pe formed by notochord in the presence of sufficient mesoderm will the norm cross-section of the tube be shown (*e*). Some of these effects have already bee seen in certain cases.

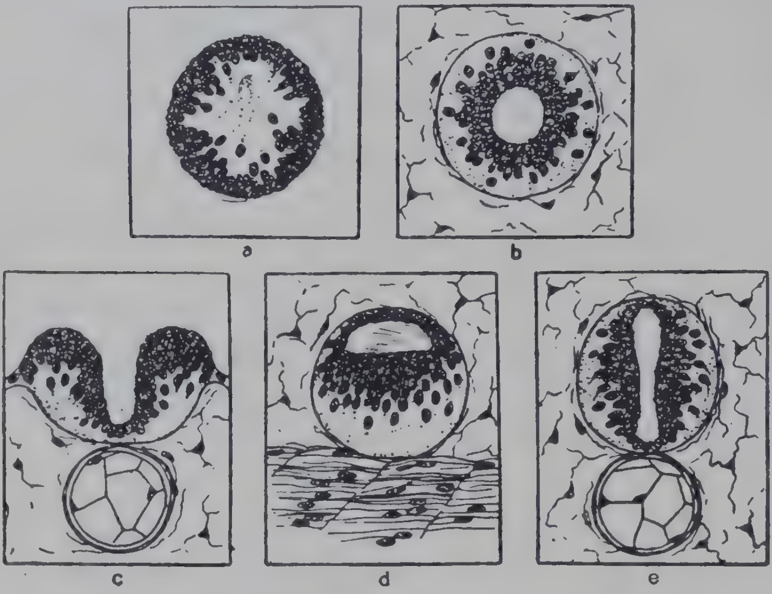


Fig. 156. Contiguity effects governing the attainment of the normal configuration of the neural tube.

In later work, Holtfreter[21] considerably extended this line of thought, developin it into a general system[22] of "tissue affinities" by the study of isolates and vario combinations of isolates. Isolated endoderm alone shows first a tendency round up into a ball of cells, and later spreads out on the bottom of the dis suggesting "intestinal" epithelium (Fig. 157; 1*a*, 1*b*). If a little ventral ectoder and endoderm are isolated together with a small amount of mesoderm, t spreading tendency is abolished, and the spherical object holds together wi endoderm forming part of the periphery and ectoderm the remainder (Fig. 157; But if more ectoderm is provided, sufficient to enclose the whole, then t endoderm will form a tubular structure within the mesoderm, thus showing th larity has been reversed (Fig. 157; 3), and simply embedding it in a mass of lerm will give the same result. It may be noted that this "gut"-formation roceeded without any prior invagination.

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* See below, p. 371, in the section on genetic factors, where failures of neural tube closure occu and for general contiguity effects, the hybrid chimaerae of Hadorn[3] (p. 359).

w what happens when ectoderm and endoderm are isolated together without lerm? At first they cling together, but later they tend to separate (Fig. 157; 4), nis self-isolation always occurs after a certain time. The converse experiment parate cultivation followed by fusion also succeeds; after a certain time, this ot be done, showing that an autonomous ageing process of "chemical entiation" or "increasing physiological incompatibility" has gone on. Thus

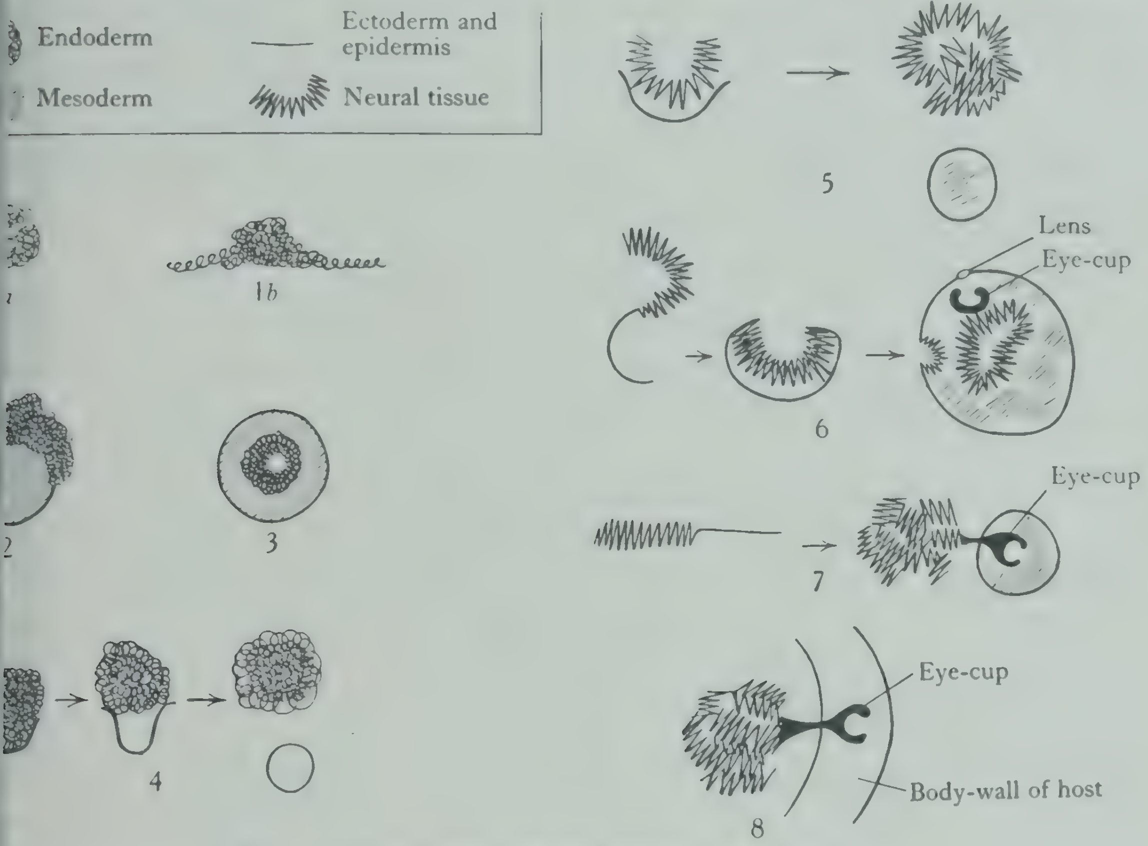

Fig. 157. Principles of tissue affinity (see text).

lerm behaves as a "connective tissue" in the truest sense of the words; ut it the building of an organism is impossible. Of the meaning and anism of these important facts nothing definite is yet known, but the problem to be essentially one of cohesion or congruence of surfaces, and urgently s attack from the point of view of surface chemistry.*

ltfreter went on to study the relations between neural tissue and ventral erm in isolate combinations. If much neural plate is combined with little erm, the epidermis will eventually separate from it, as if from endoderm, he neural tissue will form a naked brain (Fig. 157; 5). But if a better supply oderm is made available, the neural tissue will embed itself in an ectodermal in which it may induce an accessory neural plate and lenses (Fig. 157; 6). umen of the ball will fill with neural crest mesenchyme. In the former case, d an eye-cup form, the epidermis will not separate itself entirely from the neural mass, but will enclose the eye-cup, suggesting that the newly formed s organ, though not the nervous system, has an affinity for epidermis (Fig. 15 Exactly similar results may be obtained by implanting a neural mass int coelom of a tadpole; should an eye-cup be formed, it will bury itself in the b wall of the host, as if attracted by the host's epidermis (Fig. 157; 8). Ear-ve and nasal placode also "prefer" close contact with epidermis. The repul between nervous tissue and ectoderm is well seen both in normal develop and in various kinds of induction (Fig. 150), where the ectoderm seems t itself off, well away from the nervous tissue below.

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* Cf. Rashevsky[1].

All this had been adumbrated by W. Roux[6] in his theories of "cytotaxis", only Holtfreter's brilliant explantation technique could have demonstr experimentally that such effects never take place at a distance, as Roux thou but only when the tissues are in contact. It need only be added that t principles of tissue affinity are quite non-species-specific and may be see practically any desired xenoplastic combination.

2·394. Individuation and the organ-districts

What then are the successive manifestations of the individuation field? We it first in the interaction between the vitelline gradient and the grey cres cortical field, determining the point of appearance of the blastopore. At beginning of gastrulation the organisation centre acquires (rather gradually Lopaschov[4] believes) its antero-posterior regional differentiation. During induc this regional character manifests itself in the shape of the neural plate. At neurula stage it is complicated by the appearance of numerous subsidiary fi or districts each associated with the differentiation of a specific struct Lehmann's exchange experiments[2] between presumptive regions show these coming into existence. Holtfreter's grafts[5] of young gastrula tissue on to neurulae reveal, wrote Spemann ([17], p. 309), "the existence of persistent embryonic fields. In the same way as a neural field may be induced by a piece of isolated notochord in an embryo out of which the normal neural plate has already been induced, or as the same effect may be produced by a piece of neural tube, nay, even by a piece of brain from a swimming larva; thus also the whole larva, even of later stages, is permeated by embryonic fields which become manifest as soon as a piece of reacting mate has been brought under their influence. Even in the adult animal such fields h not ceased to exist; their existence can always be demonstrated by a regenerat blastema which has been exposed to their influence" (Schaxel[2]; Milojev Guyénot[2]; Weiss[7]).

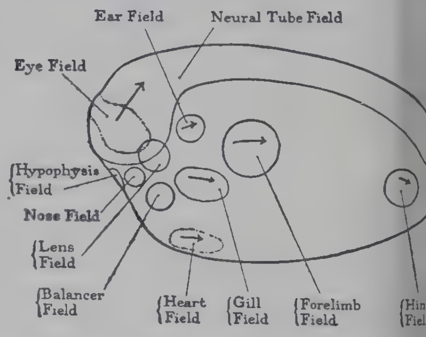

Fig. 158. The organ-districts o amphibian neurula, viewed from side. The arrows indicate dist that are polarised from the moment of their appearance (f Huxley & de Beer[2]).

Fig. 158 gives a diagram of an amphibian neurula showing these fields districts. They represent the parts of the embryo where the presump nents of eye, limb, gill, etc. will become determined. Their appearance has termed by Weiss[16] *Emancipation.* Under no circumstance can a limb be uced from a lens district, but the outer edges of the districts are indistinct, hey may slightly overlap with one another.* Here the full concept of field ity does not appear to be justified, although when the limb, for instance, has n to grow out, it may constitute a sort of field, a portion of the whole iduation field, since indifferent material added to it will be built into its form.†

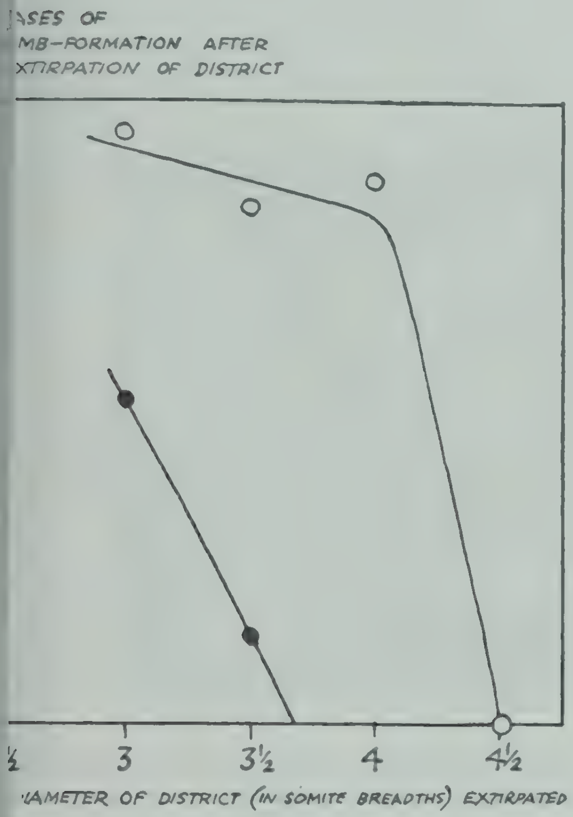

Fig. 159.

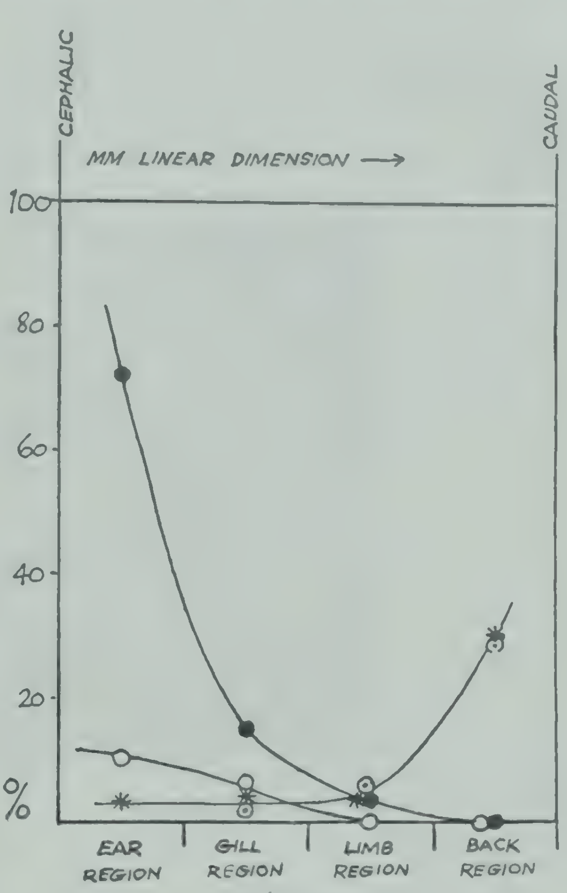

Fig. 160.

59. Relation between size of area extirpated from a district, and frequency of subsequent anifestation of its property (here limb-formation). ● Wound cleaned; ○ wound not eaned.

60. Relation between site of implantation of competent ectoderm on to the neurula, and its ubsequent fate. ● Ear; ○ balancer; * muscle; ⊙ pronephros.

e districts represent, in fact, a rather different form of order. Organ-forming cy falls off from their centres in a definite measurable way. Thus Harrison's [3] on the limb buds of amphibia will permit of graphical formulation (see 159). The percentage of positive cases of organ formation after the excision fferent sized circular pieces can be plotted against the diameter of the pieces sured in somite breadth), i.e. the distance of their circumferences from the

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the organ districts are forming, certain general determinations are proceeding, such as that count of which the ectoderm can now no longer be turned inside out and yet develop normal Luther[1]).

e of the most remarkable results of Weiss' work[11] on the regeneration of limbs is the smallness pieces of tissue which can carry the field, e.g. tiny discs of arm attached to legs produce not legs, on regeneration (see Fig. 247).

presumed centre of the limb district.* In such a case we are really measur degrees of "limb probability", and could erect probability contours which wo fix the configuration of the district round its centre. Similarly, Holtfreter[5], we have seen (p. 274), studied the results of grafting bits of presumptive epider or presumptive neural plate from a gastrula into various regions of the flank a neurula. The grafts undergo differentiation into various structures, and frequency with which a given result is obtained can be plotted against the lin dimension of the body (see Fig. 160). Here again we have essentially a pr ability measurement, namely the probability of prediction that a given point the individuation field will be found to actualise a property known beforeha to be characteristic of a given spatial part of the individuated organism. On t basis a qualitative mathematical model might be constructed. If the whole

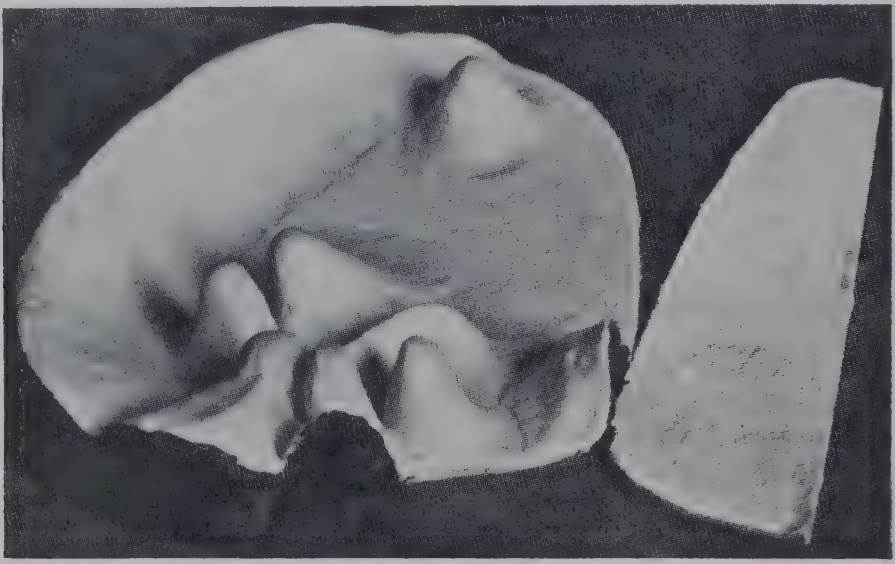

Fig. 161. Hemispherical projection of the neurula, the two halves being united at the anter part of the mid-ventral line, modelled topographically with probability as the vertical ax Key in Fig. 158.

one side of an amphibian neurula is mapped in hemispherical projection, t probabilities of organ formation can be represented by a landscape of hills, t contour lines being the "isobars", as it were, of probability (see Fig. 161). T status of any given point depends on the probability of bets that (*a*) an add piece of indifferent tissue will contribute to, (*b*) an excised piece will inhibit, t formation of such and such an organ. There must, of course, be some underlyi factor, which we could call "limb-forming intensity". Let us try to picture wh this could be.

Implicit in the foregoing discussion is the idea that each of the organ distri is associated with, is, in fact, the expression of, an inductor or a group of inducto possessing hormone-like properties.

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* The converse experiment to Harrison's, namely the transplantation of discs of varying si from the limb district to the flank, was made by Takaya. Grafts less than 4 somite breadths diameter were usually absorbed, but those of 5 and 6 somite breadths diameter produced qu normal limbs. Normal development was more likely to happen if a small piece of the do neighbouring ectoderm was included in the transplant, suggesting an asymmetry of the dist boundaries. The two sets of experiments agree well.

Huxley[13] has told us, the classical concept of hormone can now be divided everal logical subdivisions. (1) There are the metabolites with physiological ions, transported by the circulating blood, e.g. CO_2 or lactic acid. These, out possessing any high degree of specificity, certainly co-ordinate the ties of the body. (2) There are the true hormones, organic molecules ally manufactured in *ad hoc* glands, and possessing great specificity at their of action, which again they reach transported by the blood. (3) There are "diffusing hormones", such as the water-soluble heat-stable substances ribed by G. H. Parker[2]) which appear to be liberated at the end-organs of s and to control the contracting and expanding of chromatophores in fish rustacea. In this class would come the substances generated at cholinergic drenergic nerve-endings. (4) There are next the "contact-hormones", i.e. numerous evocators of different grades which determine the fates of the us parts of the embryo. The distinction between these last two classes is ery clear-cut, for the necessity of contact between the active and the passive es may only mean that the substance involved possesses very feeble powers ffusion.* Lastly (5) come substances of morphogenetic nature produced tly by genes and perhaps only acting in the cell which makes them (see on 2·67).

theoretical possibility is now of interest. It does not seem so far to have envisaged that a substance might be a hormone without diffusing at all. possible to picture a single molecule or a molecular aggregate (perhaps of acrystalline nature) exerting a polarising influence around itself in all (or) directions of space for a considerable distance, even into microscopic nsions if the argument of Hardy[2] (see p. 675) is remembered. The concentric s which would then exist would not be zones of decreasing concentration rmone (as we now generally assume) but zones of increasing randomness rangement of its own molecules or of other particles. The gradient in a given ion would not be due to the falling off of the amount of the substance but falling off of its oriented arrangement.† Limb probability might thus be ately probability of molecular orientation. But a gradient in amount of tor substance is perhaps no less likely.

cognition of the existence of organ districts in the neurula brings us to the subf the second-grade organisers, to which the following sections will be devoted. fore leaving district differentiation, however, one should note that it shows me striking examples of field fusion. The heart, for example, in amphibia, from the two lateral mesoderm plates, which, as Dwinnell has shown, may to contract some hours before they fuse. But if their fusion is prevented, each is isolated, two almost perfect hearts will be produced (Ekman[1] on hibia; Jolly on mammals).

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xley's classification actually involved the use of the term Activator; but as we have already p. 188), it may be well to reserve this for whatever it is that liberates an organiser substance nactive combination. It is unfortunate that Weiss[14, 16] also uses the term as almost synonywith Evocator.

ome such way, perhaps, as the gradual tapering off of cortex into cytoplasm in the echino- egg.

2·395. Second-grade organisers

The organisers of the second grade are so described because they come i
play after the activity of the primary organiser, the laying down of the main a
of the body, is completed. We cannot here, for lack of space, examine them
too great detail. Some treatment of them, however, is indispensable for t
reasons, first, because in certain cases the biochemical analysis of their mechani
has already begun, and secondly, because some rather important theoretical ide
such as that of "double assurance", have originated from the study of secor
and third-grade organisers.

Spemann's conception of the chains of inductors which operate in org
differentiation is illustrated in Fig. 162 (adapted from Holtfreter[19]). Neu

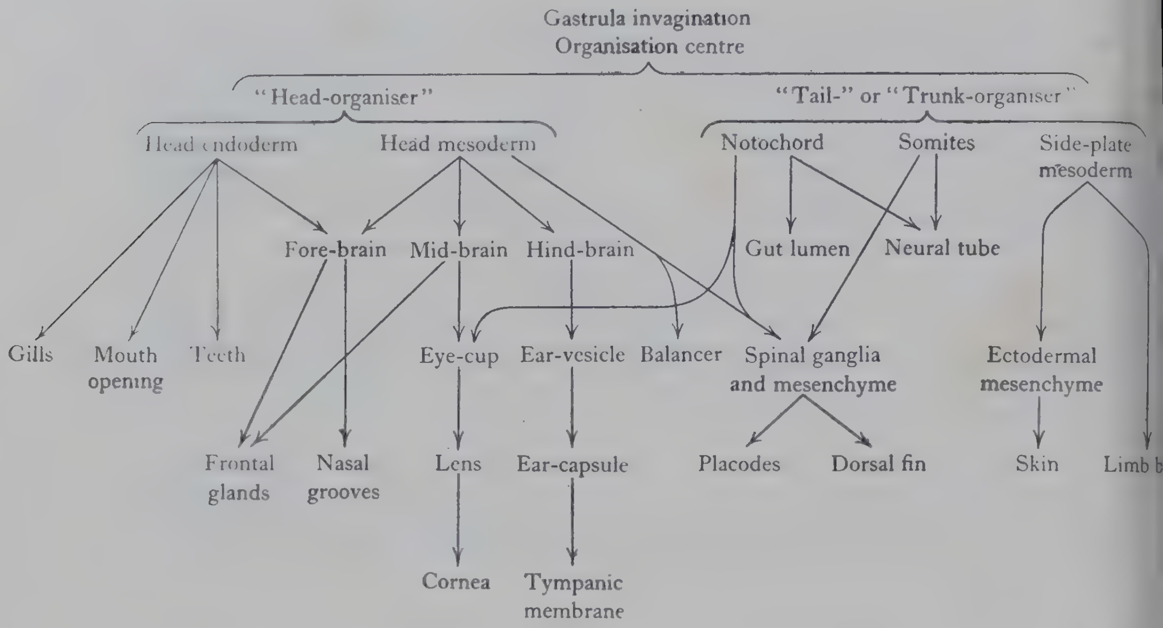
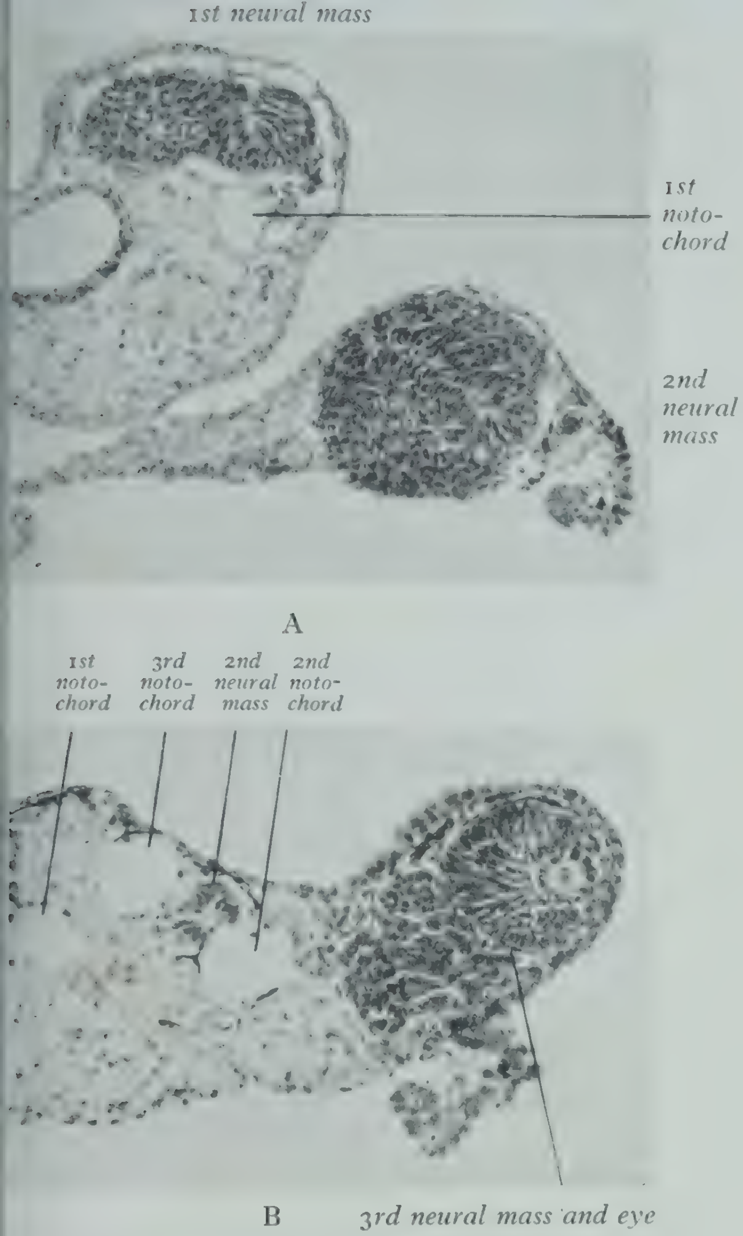

Gastrula invagination
Organisation centre
"Head-organiser"
"Tail-" or "Trunk-organiser"
Head endoderm
Head mesoderm
Notochord
Somites
Side-plate mesoderm
Fore-brain
Mid-brain
Hind-brain
Gut lumen
Neural tube
Gills
Mouth opening
Teeth
Eye-cup
Ear-vesicle
Balancer
Spinal ganglia and mesenchyme
Ectodermal mesenchyme
Frontal glands
Nasal grooves
Lens
Ear-capsule
Placodes
Dorsal fin
Skin
Limb b
Cornea
Tympanic membrane

Fig. 162. The succession of inductors of second and lower grades.

induction, as we have hitherto seen it, is shown by the action of the head enc
derm (at the junction of the archenteron roof with the yolk-endoderm) and t
head mesoderm in inducing the parts of the brain; and by the action of t
notochord and somites in inducing the neural tube. These effects are indicat
by arrows in the diagram. Second-grade organiser effects are seen in the inducti
of the lens by the eye-cup, and the ear-capsule by the ear-vesicle. The induct
of the tympanic membrane by the ear-capsule (strictly speaking, the tympa
cartilage) is an example of a third-grade induction.

An instance of a very late, e.g. fourth-grade, induction, is afforded by t
discovery that peripheral tissues induce nerve-endings of an appropriate kind
the nerves reaching them (Dijkstra; Weiss[15]).

In the present section it will be convenient to begin with the most classi
and typical case of second-grade organiser, that which operates in the format
of the lens by the eye-cup. I shall then describe shortly other cases and end
dealing with the most peculiar problem of limb-bud induction.


1st neural mass
1st noto-chord
2nd neural mass
A
1st noto-chord
3rd noto-chord
2nd neural mass
2nd noto-chord
B
3rd neural mass and eye

"Organism" formed by the development of seven dorsal blastopore lips fused together.

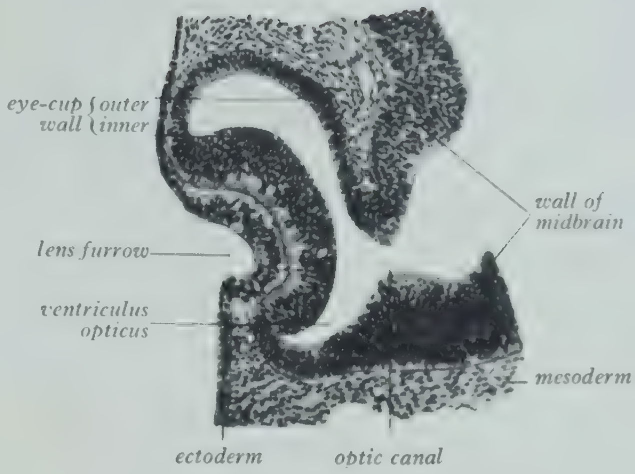

eye-cup wall {outer {inner
wall of midbrain
lens furrow
ventriculus opticus
mesoderm
ectoderm
optic canal

Fig. 163. Induction of the lens from competent ectoderm by the eye-cup.

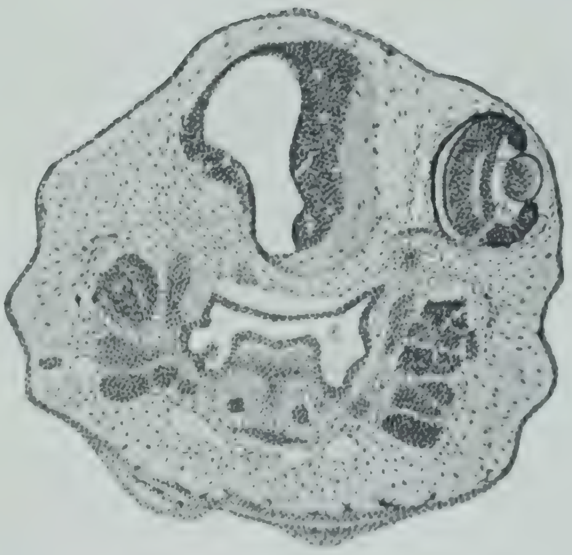
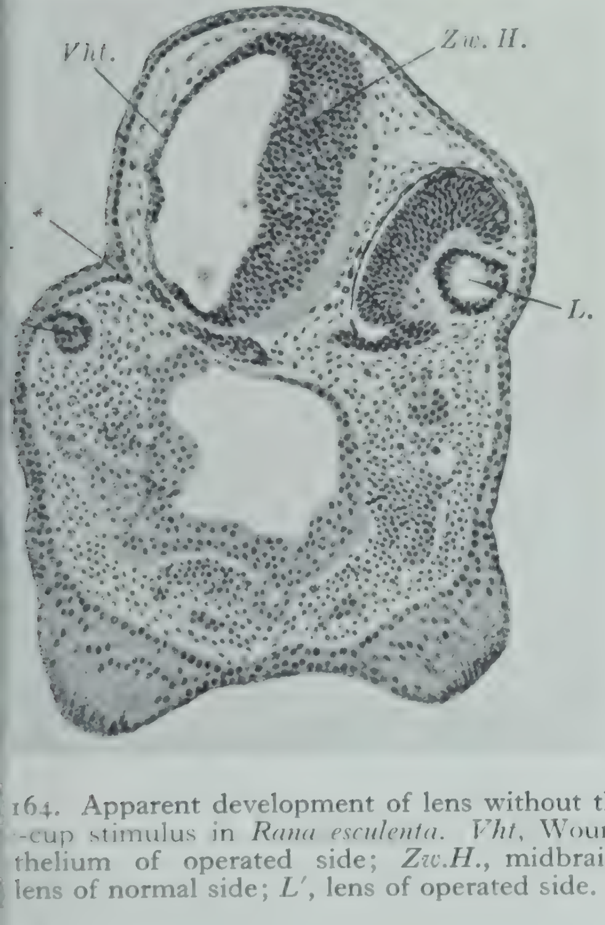


Fig. 165. Absence of lens on the operated side after removal of the eye-cup in *Bombinator pachypus.*



164. Apparent development of lens without the -cup stimulus in *Rana esculenta.* *Vht*, Wound thelium of operated side; *Zw.H.*, midbrain; lens of normal side; *L′*, lens of operated side.

3951. Eye-cup and lens induction

ıe morphogenetic stimulus emanating from the eye-cup which induces the from the ectoderm with which it comes in contact has already been referred ›. 224). Fig. 163 illustrates the process. As we have also seen (p. 105) ıann[2] showed, at the beginning of the century, that if the presumptive eye-cup nent was destroyed no lens subsequently developed, although it was certain the presumptive lens tissue itself had not been injured (*Rana fusca*). This t has been confirmed hundreds of times on many different amphibia by later stigators (see the review[8] and papers[3,4] of O. Mangold). Defect experiments followed by transplantation experiments, first carried out (independently) Varren H. Lewis[1]. The eye-cup was exposed, cut off, and pushed backwards r the ectoderm so that it would act on ectoderm never normally subjected ns-inducing influences. New lenses were excellently formed (*Rana sylvatica Rana palustris*). Filatov[6] and Woerdeman[10] subsequently showed that a stay nly 24 hours suffices for the eye-cup to exert its lens-inducing power on ectoderm. The converse experiment, that of transplanting ectoderm from ıde the presumptive lens district on to a site over the eye-cup, was also essfully made by Lewis[2]. It was thus proved that the eye-cup acts as a nd-grade organiser, inducing a lens from the ectoderm overlying it. We shall rn later to the question of the nature of the inductive influence.

ıt before long it was found that the question was not so simple as it sounds ı the above description; the historical development of the subject brought to difficulties.

In my first experiments on *Rana fusca* in 1901", wrote Spemann[17] long wards (p. 60), "lens-formation and the clearing of the deep black epidermis cornea did not take place if the eye-rudiment in the neural plate had been oved. Since a direct injury of the primary lens-forming cells could with ainty be excluded, their failure to form a lens seemed to prove that the lens ation is only possible under the influence of the eye-cup. It seemed natural tend this result to include all vertebrates. But soon cases of lens formation e complete absence of the eye-cup became known. It was shown by Mencl 03 that the lens occurred spontaneously in *Salmo*,* and after experimental val of the eye-cup by King in *Rana palustris*. Sure of my case with *Rana* and biased by the idea that all animal forms must behave in a similar way, not at first allow myself to be shaken in my conviction. The lenses in King's riment were not very distinct; Mencl's cases seemed to admit of another ınation. Nevertheless, I repeated my experiments on another object, *Rana enta*, and now it appeared to my great surprise that after a neat removal of eye-rudiment from the open neural plate, lenses of great perfection can nate...."

mann[6] was therefore forced to conclude that while in some species the lens s its origin absolutely to the stimulus from the eye-cup, in others the preptive lens district is determined in some other way at an earlier stage, and self-differentiate without the help of the eye-cup. The contrast is shown in . 164, 165, where on the one hand a lens is shown without an eye-cup in a

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* In a case of *duplicitas anterior* one head had two lenses but no eye-cups.

Rana esculenta embryo, and on the other hand the complete absence of a len
an embryo of *Bombinator*.

Later investigations have shown that amphibian embryos may be divided
several groups, according to the degree of determination of the lens district be
the eye-cup comes to act upon it. Spemann classifies them as follows:

(1) A highly differentiated lens develops from the lens district without
assistance of the eye-cup:

Rana esculenta	Spemann[6]

(2) Ectodermal thickenings or vesicles of questionable significance deve
from the lens district without the assistance of the eye-cup:

Rana palustris	H. D. King
Bombinator pachypus	Spemann[4]; v. Ubisch[3]
Rana fusca	v. Ubisch[3]
Rana catesbiana	Pasquini[2]

(3) Absolutely nothing takes place in the lens district without the assista
of the eye-cup:

Pleurodeles waltlii	Pasquini[1]
Triton taeniatus	O. Mangold[3]
Triton alpestris	O. Mangold[3]
Pelobates fuscus, *Bombina bombina*, *Triton cristatus*	Popov, Kislov, Nikitenko & Canturisvili

The second and third of these groups may for the present purpose be tak
together, as it was in *Rana fusca*, after all, that the first proof of the eye-c
induction was obtained. The cases of *Rana esculenta* and to a lesser extent
Amblystoma punctatum are the real difficulties.*

Two other peculiarities have been noted which differentiate these two spec
from the others in the list, first the lack of dependence of the size of the lens up
the size of the eye-cup, and secondly, the distribution of lens competence over
neurula ectoderm. With regard to the first of these, Spemann[17] has observed t
in *Rana esculenta* a diminution in the size of the eye-cup does not bring ab
any corresponding diminution in the size of the lens, whereas in a species su
as *Bombinator*, where the formation of the lens is very highly dependent up
the eye-cup, we find that the smaller the eye-cup or piece of eye-cup, the sma
the lens. The detailed references on which these facts are based will be found
Spemann's book ([17], p. 67). This property fits in quite well with the degree
inductive dependence shown by the lens district. With regard to the seco
peculiarity, embryos of different amphibian species differ in the extent to wh

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* Spemann[17] lists *Amblystoma punctatum* also among those forms in which a highly differenti
lens develops from the lens district without the assistance of the eye-cup. But this is not altoge
convincing, for the following reasons. Le Cron showed that removal of the eye-cup resulte
failure of lens formation. Then Harrison[4] transplanted presumptive lens-district ectoderm to o
regions of the head and found that well-formed lenses resulted. But before removal this ectod
had been for a short time in the near neighbourhood of the eye-cup. Harrison wrote: "Con
between eye-cup and ectoderm has at this time been established, though the two are not adhe
and may be readily separated without cells from one layer sticking to the other." One has
to assume therefore that in this species the action of the lens-inducing substance is particul
rapid. If the lens ectoderm was removed in earlier stages "small and not fully differenti
lenses" sometimes but not always resulted. The form belongs therefore rather to group (2).

forming competence is distributed over the ectoderm. In *Rana fusca** it is where. But in *Bombinator*, though head ectoderm from any site will form trunk ectoderm will not. And in *Rana esculenta* lens-forming competence tricted completely to the presumptive lens district (Spemann[5]).† This again with the degree of inductive dependence shown by the lens district.‡

the other hand, the eye-cup of *Rana esculenta* is undoubtedly giving off orphogenetic stimulus, as was shown by the experiments of Filatov[2] and uini[2]. Ventral ectoderm from a species with widely extended lens competence *vulgaris*) was transplanted over the eye-cup of *Rana esculenta*, with the result an excellent lens was produced. We know that without such a stimulus this erm would never have reacted in this way. The paradox thus arose that the cup of *Rana esculenta* appears to give out a stimulus which its ectoderm s in no need of.

is fact has been the principal mainstay, though not the first origin, of the eption of "double assurance". This interesting idea, taken from engineering, first used by Rhumbler[1] in his mechanical descriptions of cell-division about (*mehrfache Sicherheit*), and was used by Spemann[12] for the present case other cases in embryology (*doppelte Sicherung*). Just as an engineer plans dge to carry loads and to withstand wind pressures much larger than any h it will normally meet with, so the forces of evolution have brought it t that some ontogenetic processes involve more than one chain of causation. may mean (1) that in the event of one process failing, another one, previously serve, will accomplish the same end, or (2) that normally two or more esses collaborate towards the production of the same result.

emann (p. 92)[17], believes that the principle of double assurance is well lished, and that it is of far-reaching importance, not only in development, also in the functions of the adult organism. In its first form, as stated above, ay lead to strange conclusions. Some biologists have not failed to find it r bizarre that nature should have taken such elaborate precautions through ages, in the case of *Rana esculenta*, against the operative methods of e experimental embryologists. It is true that nature does many strange s; thus we have seen already many inductors with nothing to evocate, and instances of slumbering competence which is never awakened by the opriate inductor. In its second form, however, it is with difficulty gainsaid. can doubt, as F. E. Lehmann's[4] "synergetical principle of development" *binative Einheitsleistung*) asserts,§ that very many causes must have contributed towards the normal configuration of a perfect neural tube or a perfect But nevertheless it is always our duty, in conformity with the immortal

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d in many other forms, e.g. *Triton vittatus* (Sikharulidze).

t confirmed by Gosteieva on the Russian variety of *Rana esculenta*; a varietal difference? also the work of Manuilova[2].

t according to Popov[1] these differences are only quantitative. Body ectoderm of *Bufo viridis*, h, when tested by implantation of eye-cups under it, has no lens competence, will, when nted into the eye-cup cavity as for Wolffian regeneration experiments (see p. 295), give a

also Bytinski-Salz[3].

principle of William of Ockham, to search for the simplest possible causat chains. And I am not convinced that the principle of double assurance has yet, far as the development of the eye is concerned, been put on a basis quite f from criticism.

There are two ways in which the anomalous behaviour of the lens district *Rana esculenta* could be explained so as to bring it within the realm of straig forward inductions. It could be said that the presumptive eye-cup region, ev while yet still undifferentiated from the anterior part of the neural plate, possess in these species lens-inducing power, so that the determination of the le though occurring very early, was due to the eye rudiment all the same. This w the suggestion of de Beer ([1], pp. 183 ff.), but Spemann ([17], pp. 94 ff.), perha justifiably, regards it as unconvincing, since no parallel for such an "inductio at-a-distance" is known and he himself had shown that even a thin layer mesoderm lying between eye-cup and competent ectoderm would block t passage of the inducing stimulus.

The other suggestion is a new one. Let us suppose for the sake of argume that the inducing stimulus is mediated by a definite chemical substance (for fac bearing on this, see p. 298). In most species this substance would be sharp restricted to the eye-cup itself, but in some, such as *Rana esculenta*, it mig escape from the eye-cup by diffusion or otherwise—at any rate it might present in the mesoderm surrounding.* It this were so, removal of the eye-cu would not imply removal of all of the effective substance, and a lens wou develop later on, giving the impression that it had self-differentiated. The cruci experiment would therefore be the cultivation of *Rana esculenta* presumpti ectoderm in true isolation, as for instance in Holtfreter solution. If the theo of double assurance is right, a lens should be produced even in these condition but if the material stimulus is necessary, there should be no lens. When this te was made by Perri[2] in 1934 with numerous explants, no lenses resulted. *Bombinator* isolation experiments were also carried out by v. Ubisch[3]; in this ca two-layered vesicles with no sign of lenses were formed. Harrison's tran plantations[4] of presumptive lens district into the head ectoderm of other axolo larvae do not help here, since the new environment was not strictly neutra But strangely enough, even *Rana esculenta* lens districts under such conditio did not develop lenses.†

Unless, therefore, Perri's crucial experiment of keeping presumptive le district ectoderm of *Rana esculenta* in isolation from an early stage onwar should be repeated and found to be wrong, the theory of double assurance mu

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* A parallel to this could be found in the anti-coagulation substance, heparin, allied to chondroi sulphuric acid (see p. 443), which is found in the tissue around the great blood-vessels (Wilande

† After I had written this paragraph I found that a similar idea had been mooted by othe Thus Mikami[3] points out that in some cases head mesoderm has been shown to possess le inducing power (Okada & Mikami). He himself found that on transplanting presumptive le district to other sites, lenses were only formed in the head and gill regions, not elsewhere. Ho freter[13], too, takes the same point of view, since in some of his xenoplastic transplantations done quite other reasons, lenses arose without eye-cups in the close neighbourhood of other he organs ([13], p. 404). Only by explantations into neutral medium can the problem be fina settled.

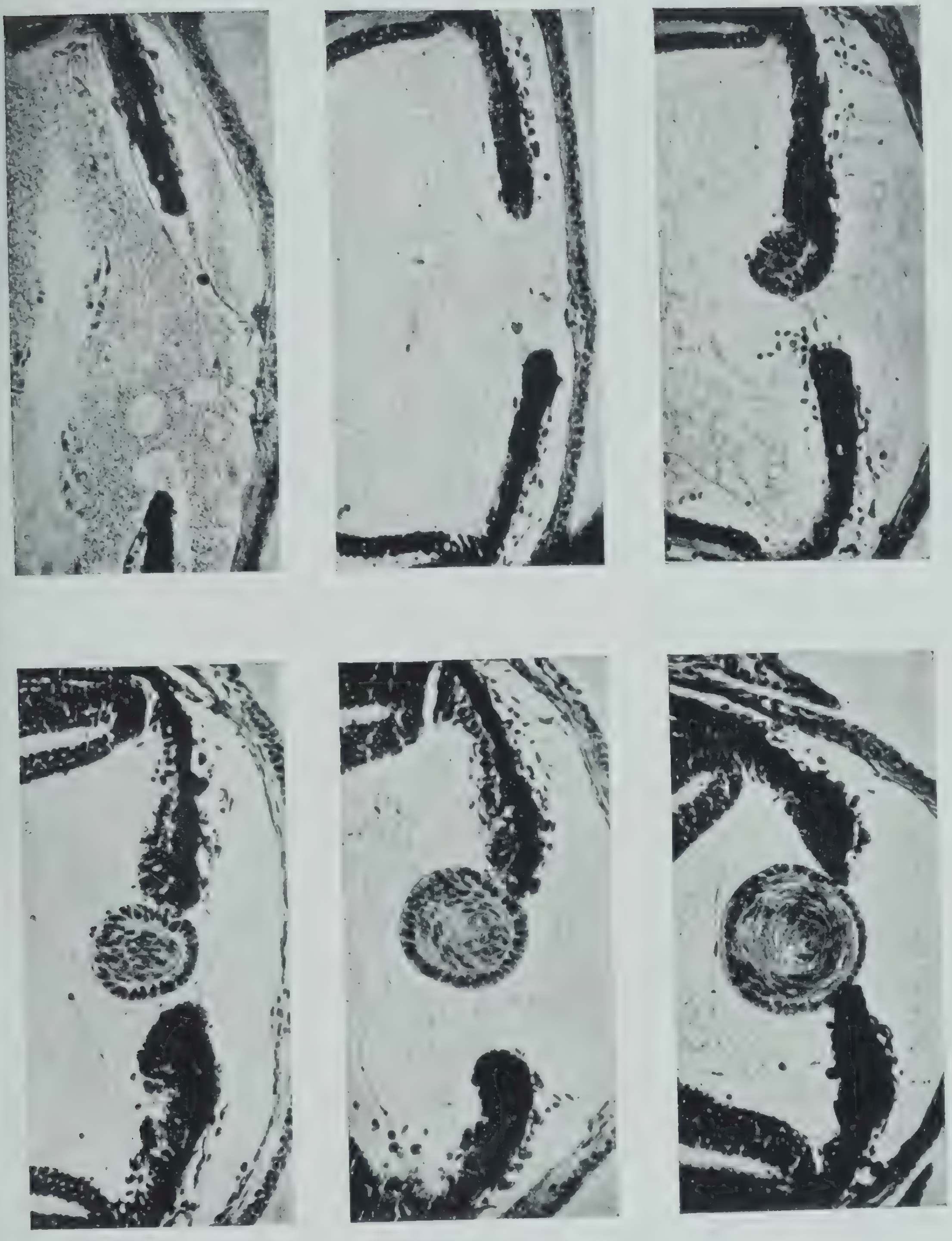

Fig. 166. Wolffian regeneration of the amphibian lens from the margin of the iris.

eld to be disproved. Its application to the primary organiser (in the work oerttler[1]) has been generally abandoned (see p. 157), and reasons will later iven for doubting its applicability to the opercular perforation (see below, 08), and the induction of the ear (p. 302).

Fig. 158 we have seen the approximate position of the lens district in the ula, but we know also that lens competence, in most species at any rate, is h more widely distributed over the ectoderm. It appears also to be present me strange places. It is to be found in the last place, perhaps, where it it have been looked for, namely the eye-cup itself. In the nineties of the last ury the discovery was made independently by Colucci and by G. Wolff that larval or even in adult amphibia the lens is removed, a new one will form the upper margin of the iris ("Wolffian regeneration"). The beautiful series ctures taken from T. Sato[1] show this process in operation (Fig. 166). The new usually arises from the upper margin, whether or not the eye has been iously turned upside down (T. Sato[3]), but if this operation is performed later then the new lens regenerates from the lower margin (the previous upper , as Wachs[2] has shown. In other words, competence for lens formation ists into adult life in the iris, but only in a half-segment of it, and the area is persistence of competence is itself determined when the dorso-ventral axis e eye is determined. But this competence of the eye-cup may under certain imstances come into play even in early development, as it did in Spemann's riment[3] where mesoderm intervened between the eye-cup and the ectoderm, that of Beckwith, where the lens competence was absent from the covering derm,* or in that of Perri[2], where eye-cups were explanted *in vitro* without ectoderm.

olffian regeneration led to a method of study of the nature of the inducing ence which has not been without results. At Spemann's suggestion Wachs[1] ff small pieces of the iris and pushed them into the eye-chamber (the *corpus m*); there, quite without contact with any tissue, they were induced to form shaped lenses. Later on, the same experiment was successfully made with s of ectoderm by T. Sato[1] and by Nikitenko[1], Wachs[1] having previously olled the process by finding no change when pieces of iris were implanted labyrinth of the ear. According to Törö[1], iris tissue cultures can be converted lens (chick). If the results of Popov[1] are to be accepted, lens competence is widely distributed. In some chance cases he noticed[3] lentoidal bodies prod in mesoderm and even in endoderm adjacent to implanted eye-cups of *esculenta*, so he implanted neural plate tissue, eye-cup itself, ectoderm, te mesoderm, and ear-vesicle into the eye from which the lens had been oved[6]. All of these formed lenses (eye-cup and ectoderm not surprisingly), the exception of the ear-vesicle. But the relations of the eye-cup with the vesicle seem to be rather peculiar, for Dragomirov[1] found that eye-cup will

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e possibility that lens formation over transplanted eye-cups might really be by Wolffian eration and not from the belly ectoderm was examined in an *ad hoc* investigation by nann[2] and ruled out, though such Wolffan regeneration is possible if the eye is placed so as to nwards (see also Mikami[5]).

induce lens from ear-vesicle instead of ectoderm if it should come into contac with it. Ear-vesicle, too, according to Dragomirov[4], may be diverted to form retina (Figs. 167, 168, opp. p. 300). Lens competence in epidermis seems to las into very late stages in some amphibian larvae (Popov, Eudokimova & Krymova and it has been shown by Schotté & Hummel to exist in both ectoderm and mesoderm of the regenerating limb-bud blastema* (see also p. 442). Durin metamorphosis there is temporary decline in the capacity for Wolffian regeneration (Monroy[2]).

It is interesting that Wolffian regeneration fails in the presence of a norma lens. Although a chemical stimulus must be involved, no induction takes plac if a piece of iris is placed in an eye-cup which retains its lens (Wachs[1]). Kobayash tried to influence lens regeneration by injection of lens-protein into the circulation but unsuccessfully. T. Sato[4], however, threw further light on the problem b finding out exactly how far lens regeneration from the iris had to go before i could inhibit the formation of a lens from a piece of iris or ectoderm implante into the eye-cup. The results are shown in Fig. 169. During the first four day after lens extirpation no change is seen in the iris and during this time norma regeneration from implanted iris fragments was quite uninhibited. On the fift day, however, the first sign of Wolffian regeneration is seen in the depigmentatio of the iris; this coincides with a great reduction in the number of fragments whic give a lens. At later stages, regeneration from fragments entirely fails, and suc fragments often differentiate into retina-like tissue.

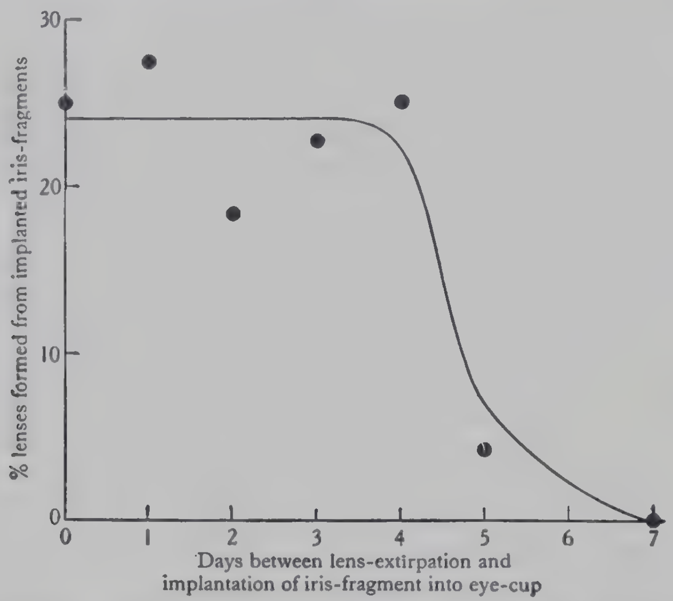

Fig. 169. Relation between degree of development of a lens arising by Wolffian regeneration, an its power of inhibiting lens-formation from an implanted iris-fragment.

Can a dead lens exert the inhibitory action? From the work of Kesselyak i appears not, so a protein is indicated as the active factor rather than a smal molecule insensitive to denaturation.†

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* Contested by Stone & Sapir. Schotté[6] has replied and the question is not yet quite settled.

† About all we know of the embryology of the lens-proteins is that the characteristic β-crystalli does not appear till the first differentiation of the lens-fibres (Sauer[2]).

olffian regeneration also fails normally in some amphibia. In the Oriental . *Hynobius unnangso*, for example, it does not occur, but Ikeda[1] showed by dding eye-cups in mesoderm that it is nevertheless possible in very early s. It is not normally seen because the induction of the lens in the ectoderm much faster process. Competence for lens or lentoid formation appears l parts of the eye-cup simultaneously but at different strengths. Like omirov, Ikeda found that structures other than ectoderm or eye-cup , such as the nasal placodes, possess lens competence and perhaps the lens ctor.

e significance of the induction of lenses from small fragments of iris or other e implanted into the eye-cup is, of course, that they are lying free there, and the lens-inducing substance must therefore be diffusing across a considerable nce of vitreous or aqueous humour. Mikami[1] has shown that the exact

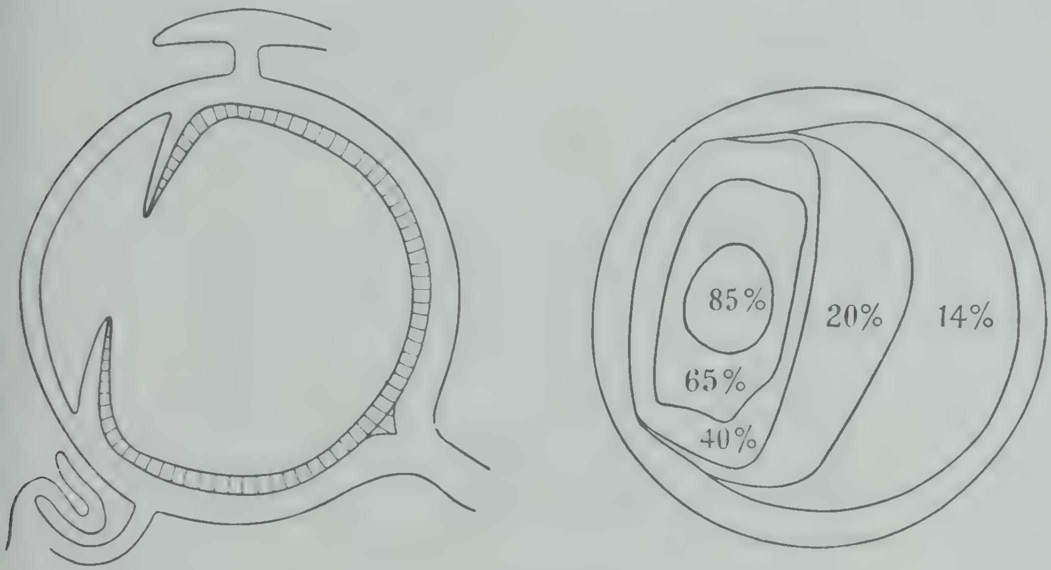


Fig. 170. Topographical distribution of frequency of lens-formation from implanted iris-fragments.

ion of the implanted fragment is a matter of some importance. If a sagittal on of the eye be compared with a frequency contour map based on the ber of successful lens inductions obtained, it is seen that the most favourable ion is about that of the normal lens itself, though either in front of it or way behind it are favourable positions, while the furthest recesses of the y are not very favourable (see Fig. 170). This may be a hint regarding the n of the inducing stimulus.

e may now turn to the experiments with injured and killed eye-cups. žaiev[5] (following the example of Spemann[14] and Krämer) subjected eye-cups rushing between two glass plates, then implanted them under flank ectoderm he late gastrula. Sometimes a fairly well-formed eye-cup would regulate lf out of a small piece which had escaped the crushing; in this case, a lens ld be induced, but no lenses were induced by the squashed material if lation failed. On the other hand in two cases ectodermal thickenings, possibly y stages of lens formation, were induced after implantation of a heat-coagulated eye-cup. Implantation of boiled mouse liver gave negative resul Similar experiments were made, in larger series, by Popov & Nikitenko, but th too never obtained true lens induction from killed eye-cups implanted in gastrulae.

Lopaschov's experiments[5] were more systematic. They consisted in the implantation of from one to seven eye-cups, living or heat-coagulated, either int the blastocoele or into isolated ectodermal balls. In the blastocoele even five livin eye-cups induced only one lens. Neural inductions were found only in th isolated balls. A single dead eye-cup gave no induction, but two to seven alwa induced atypically shaped eye-cups; these then induced lenses from the ectoderm

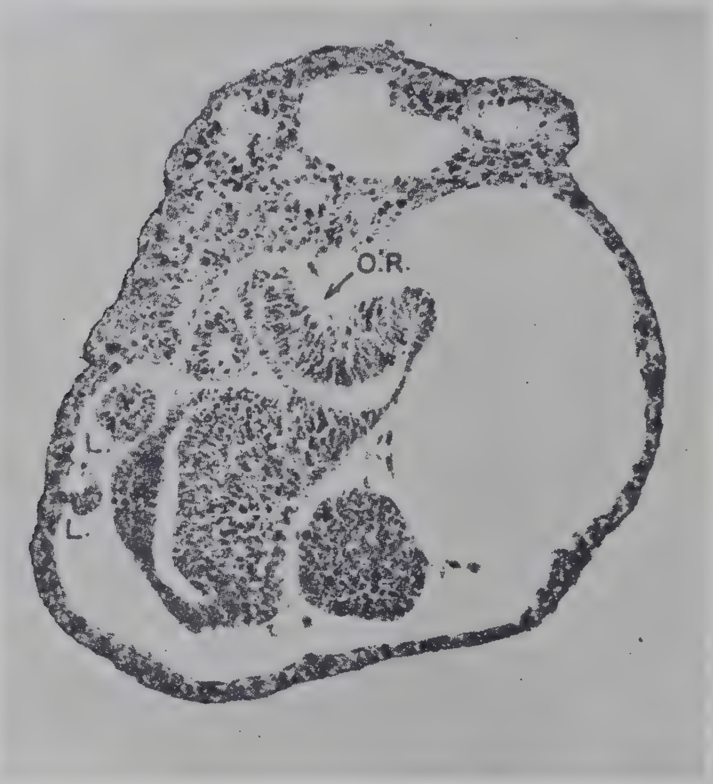


Fig. 171. Imperfect lenses induced by eye-cups themselves the result of neural inductions performed by dead eye-cups on competent ectoderm. O.R. eye-cup; L, lentoids.

In one experiment seven dead eye-cups produced three small eye-cups. The result suggested, therefore, that not only was the lens-inducing substance present i the killed tissue, but also an eye-cup-inducing substance, perhaps present in th neural plate as soon as it arises, and distinguishing (among other things) head organiser from tail-organiser. This point has already been mentioned (p. 278 The hypothesis of an eye-cup-inducing substance, emanating from head mesoderm or from presumptive brain tissue, already discussed by O. Mangol ([8], p. 232), was elaborated by Lopaschov[7] in a later paper. He took head mesoder from an early neurula, allowed it to fuse with a piece of hind-brain (the eye-cup derive from the mid-brain), and surrounded the mass with a piece of bell ectoderm. In a considerable number of cases excellent eyes resulted. This i l evidence for the existence of a specific eye-cup-inducing substance in the l mesoderm.

he subject was further pursued by C. D. van Cleave and by Mikami[2]. The sensi-y of van Cleave's material (*Amblystoma punctatum* instead of *Triton taeniatus*) not permit him to implant more than one boiled eye-cup into one ectodermal . In most cases no effect was produced, but in some there was a differentiation ie ectoderm into neural masses with cavities, and in three cases a new eye-cup formed which induced a lens in the ectoderm near it (Fig. 171). Ethereal and one extracts of eye-cups, implanted in egg-albumen into ectodermal balls, : no neural inductions and no lens formation, but the experiments were not numerous or varied. Mikami had better success (see below).

he question of the chemical nature of the lens inductor and the eye-cup ictor therefore stands now in about the same position as the question of the ire of the primary evocator stood in 1931. It invites much further research. : will be noticed that in most of these experiments with dead eyes, lenses were usually found unless an eye-cup itself had first been induced. This may mean the lens evocator is less stable to boiling than the eye-cup evocator. One interesting fact which bears on this is the induction of solitary lenses, lenses without any neural or eye-cup-like structures, brought about by the lantation of adult tissues into the blastocoele cavity (Holtfreter[12]; Toivonen[2]). . 172 shows two such solitary lenses produced by the implantation ectively of fresh salamander liver and boiled salamander heart. These ervations, though incontestable, have given rise to much misgiving in the ıds of those who have sought to unify facts and hypotheses in this field. Thus mann ([17], pp. 220 & 370) has commented: "One thing is evident from the inning, that a cooked heart, for instance, which induced an isolated lens, not have brought about this effect by some specific lens-forming agency." v the simplest assumption is, as we have seen, that primary organiser action lves a chemical substance, and that since all adult tissues act, they must ain it, capacity to react to it having long been lost. If they contain the primary ator, why not the secondary ones also? The disinclination to postulate a iderable number of active substances is, I believe, misguided, for there is ick of chemical compounds isolated from living tissues for which no function ever been found or even suggested.* It is therefore not evident that coagulated t tissue cannot contain the lens-inducing substance.

oltfreter's results were afterwards confirmed by Okada & Mikami, who id, however, that the lenses produced after implantation of heart and r were slightly atypical in form and structure. Nose rudiment, head soderm, and neural plate or brain also have the power of inducing lenses, protein-denaturation (boiling or alcohol) does not destroy it (Mikami[4]).

Inositol is an excellent case in point. A universal cell constituent, synthesised by the chick ng its development (*CE*, p. 1229, 1240), it had been known for more than half a century, no function had ever been attributable to it (cf. the review of Needham[1]) till Eastcott's dis-ry that it is one of the components of the Bios group of phytohormones (see p. 214). Another llent case is kynurenin (see p. 410). The general views here adopted are in sharp contrast iose recently expressed by Woerdeman[9].

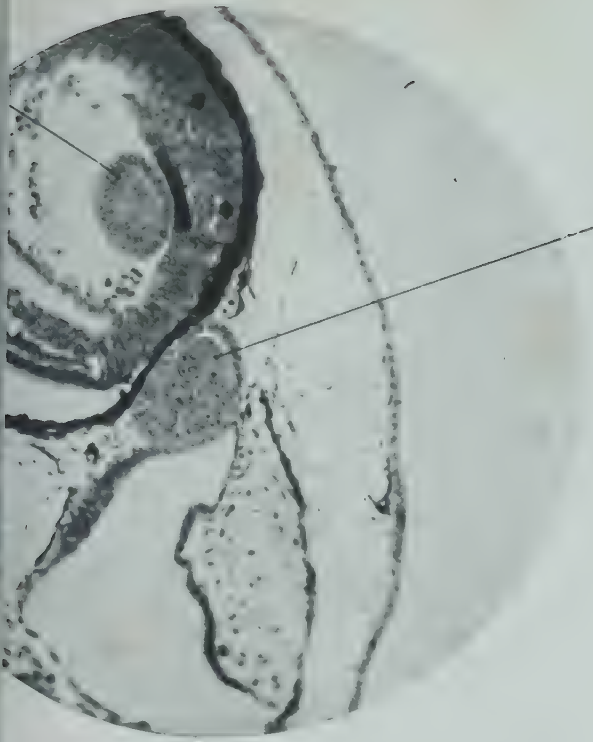
If the phenomenon of lens induction is due to a chemical substance, it migh be possible to exhaust the store of it in the eye-cup by repeated extirpations the lens followed by successive regenerations. This interesting experiment ha been carried out, and it was indeed found that the third lens of such a series wa very ill-developed, after which the power of further lens formation fell to zer (Manuilova[1]; Manuilova, Machabeli & Sikharulidze). But after a prolonged rest the eye could again form a lens from inserted material. Development of th power of restitution (synthetic formation of the substance?) therefore require a period of time. Eye-cups of different species seem to have different stores, fo Nikitenko[2] and Ciaccio obtained regeneration seven times in succession.

Of the metabolism of the eye-cup during lens induction practically nothin is known. The only hint arises from the work of Woerdeman[3], who, stimulate by what he had seen in the dorsal lip region after invagination (p. 190), examine the eye-cup histochemically for glycogen. During lens induction the central par of the eye-cup was found to lose its glycogen almost entirely. J. Mori states tha the retinal portion is early enriched with cholesterol esters. Much further wor will be needed to elucidate the meaning of this, and to establish the metaboli changes going on in the eye-cup.

As the eye-cup is an outgrowth from the neural axis, and as new neural plate may be formed by the action of the latter in homoiogenetic induction (p. 154) it might be thought that eye-cups implanted into the blastocoele would induc secondary neural axes. This, however, when specially tested by Schmidt & Ragozina and by Holtfreter[7], was found only to occur extremely rarely, if at all

Before leaving the subject of lens induction, we must refer to a question o much theoretical importance which work on lens induction has settled. It may be asked whether a tissue which has never come under the influence of the primary organiser can react successfully to that of a secondary organiser. Coul isolated ectoderm taken from the ventral region of the early gastrula, for instance react to a lens-inducing stimulus? Although it would have held a certain positio in the pre-gastrular individuation field (Dalcq & Pasteels), it would never hav suffered the remote effects of primary neural induction, and would never hav felt the quite powerful influence of the individuation field of the late gastrul and early neurula stage.

The question was answered by three investigators using three differen methods. Waddington[13] isolated presumptive ectoderm from the young gastrula cultivated it for some time, and then implanted into it presumptive eye-cu material from older embryos. Well-formed eye-cups resulted, together wit lenses from the ectoderm (see Fig. 173). Filatov[8] implanted eye-cups into *Bauchstück*, i.e. an embryo from which the organisation centre had previousl been removed and which was nothing, therefore, but a mass of endoderm cell surrounded by a layer of ectoderm. Here again, though no previous neura induction had occurred, lenses were induced. Woerdeman's procedure[8] was mor complicated than either of these; he implanted eye-cups into the flank of a lat neurula as far away as possible from the head ectoderm, and then grafted ove these eye-cups ectoderm of various ages from much younger embryos. It wa



7. Induction of lens from the wall of the ear-vesicle by the eye-cup.

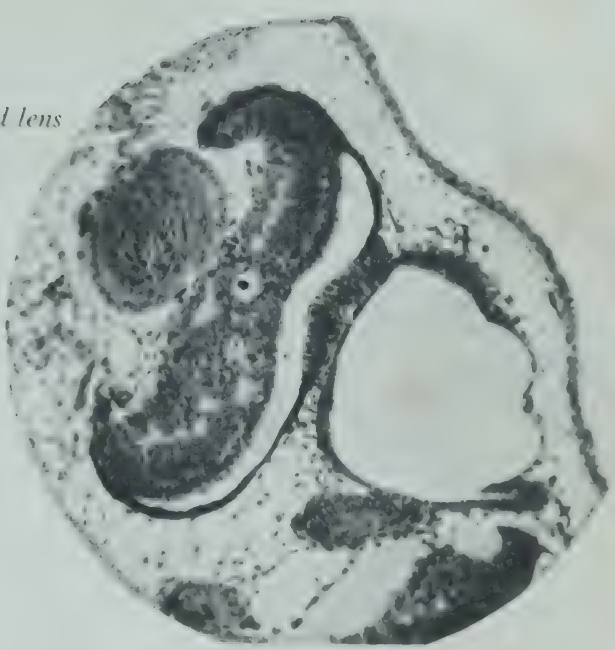
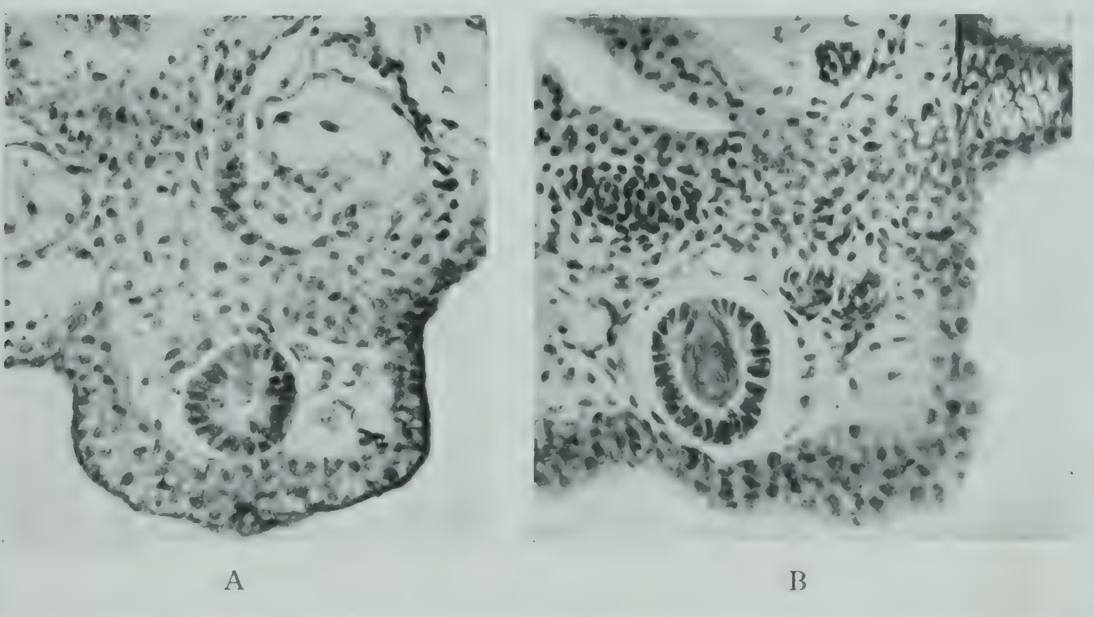


Fig. 168. Diversion of the wall of the ear-vesicle to form additional retina.



g. 172. Solitary lenses (without eye-cups) induced from ectoderm by implantation of adult tissue into the blastocoele cavity. A. Salamander liver (fresh). B. Salamander heart (boiled).

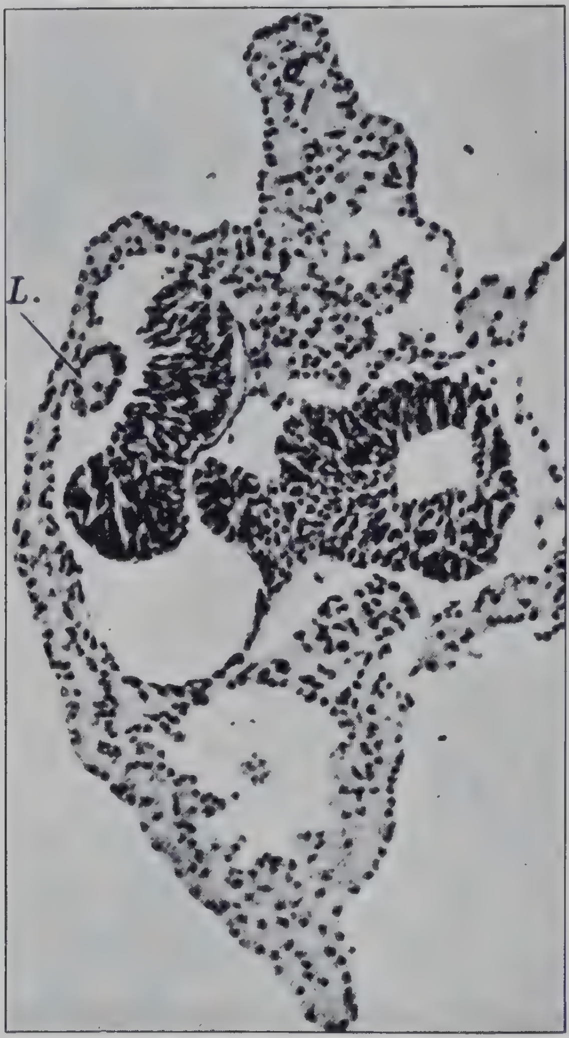


Fig. 173. Induction of a lens (*L.*) from ectoderm which had never been subjected to the gastrular individuation field.

d that ectoderm from the youngest gastrula stages would form lenses. There therefore be little doubt that competence to react to a secondary organiser ulus arises before the embryo has been stabilised by the effective action of primary organiser.

3952. Other second-grade and third-grade inductors

e shall have little to say about other second-grade and third-grade organisers, p to the present time little progress has been made with them from the nical point of view. Much information regarding them will be found in the ter on self-differentiation (the "mosaic" phase of development) in the book of ley & de Beer[2]. A list of them (which makes no claims to be either complete nal) is given in Table 22; it provides a rich field for the biochemistry of the re.

nder the head of second-grade organisers we ought perhaps to include the nical stimuli which have been believed by some to underlie the phenomena eurotropism, i.e. the attraction of outgrowing nerve-cell axons in specific ctions. Little work has been done in this field recently, though before 4 it was much studied by Ariens Kappers and Ramon y Cajal; reviews up to 3 by Tello and O. Mangold[5] should be referred to, together with Harrison's onian lecture[14].

large amount of work has been done on the determination of the cornea; ill be found in the review of Mangold ([8], p. 294). Popov[2] has shown that ea competence is extremely widespread. All the workers are agreed that the ulus from the eye-cup is chemical, though mechanical extension of the skin an implanted ball of glass (W. H. Cole) or wax (Baurmann) has a slight effect. t this is due to the curvature was shown by Cole, who obtained nothing at vhen a flat piece of glass was implanted under the skin.

he inductor for the blood (see pp. 164, 378, 648 ff.) is not definitely known, t forms in a small region just anterior to the heart, and if this is extirpated, pletely bloodless larvae can be produced (Frederici; Słonimski). Remarkable ers of blood-vessel construction are shown by the blood cells, to judge from bservations of Parker[3] and Hueper & Russell on the formation of capillaries. rtilage of the visceral skeleton is formed from the mesenchyme of the neural ts (Stone), which possesses a power of neural induction never normally loyed (Raven[2]), since it meets no competence in its migrations. According ischer[3], cartilage can make more of itself from added fibroblasts, and so should egarded as possessing a specific inductor. This could not be confirmed by njantzev & Berezkina (see below, p. 438).

he formation of the ear-vesicle, it is known, depends on a stimulus from the hind-in and head mesoderm. From the work of Filatov[1]; Guareschi[1], Lopaschov[7] others it is known that the formation of the cartilaginous auditory capsule ependent on a stimulus from the ear-vesicle. Like most secondary inductors, is quite non-species-specific (Albaum & Nestler; G. A. Schmidt[7]). But other ctions also take place before the final state of the auditory system is reached. bably stimulated by thyroxin, the metapterygoid region of the quadrate buds

Table 22. *Second and lower grade organiser effects*

Induced structures	*Inductor*	*References*
SECOND-GRADE ORGANISER EFFECTS		
Eye-cup	Mid-brain and head mesoderm	Lopaschov[7] (1); Adelmann[3]; Alderman
Lens	Eye-cup	Spemann[2]; W. H. Lewis[1]
Nasal placodes	Fore-brain	Raven[2]; Lopaschov[6] (1); Kawakam
Ear-vesicles	Hind-brain and head mesoderm (6)	Dalcq[2]; Guareschi[5]; Mangold[16]; Harrison[13] (2); Lopaschov[6] (1); Ponomareva[1]; Gorbunova; Kogan
Posterior pituitary	Notochord (anterior end)	Bedniakova
Gills	Branchial endoderm	Ichikawa[2]; Severinghaus; T. H. Shen; Mangold[13] (3); Lopaschov[6] (1)
Spinal ganglia	?	Harrison[18]
Pronephros	Mesoderm	Machemer
Limb buds	Mesoderm	Mangold[16]
Teeth Mouth-invagination	Pharyngeal endoderm	Ströer; Riesinger (4); Holtfreter[14]
THIRD-GRADE ORGANISER EFFECTS		
Dorsal fin	Spinal ganglia mesenchyme	Harrison[7]; Holtfreter[19]
Cornea	Eye-cup (all parts) and lens	Mangold[8]
Retina	Eye-cup	Dragomirov[5]
Ear-capsule	Ear-vesicles	Filatov[1]; Guareschi[1,2]; Kaan
Tympanic membrane	Annular cartilage from quadrate	Helff[6]
Heart and blood	Endoderm	Balinsky[10]
Suckers Balancers	Fore-brain and fore-gut endoderm	Lopaschov[6] (1); Holtfreter[19,20] (7) T. Yamada[1]
Anterior pituitary	Posterior pituitary and brain (8)	Gaillard[3]; Burch; Eakin[2] (10)
Sense organs of lateral line	Brain or ear-rudiment	Harrison[1] (5); Stone[1]
Anus	Tail-bud mesenchyme	Risley; Schechtman[8]
Ciliary polarity	Mesoderm	T. C. Tung & F. Y. Tung[1] (9)
Mesonephros (13)	Pronephric (Wolffian) duct	Waddington[17]; Grünwald; O'Connor[1]
Cloacal diverticula (chick)	Pronephric (Wolffian) duct	Boyden[1]; O'Connor[2]

Notes to Table 22

(1) This was assured by Lopaschov's experiments[6] in which the whole of the neural plate of young neurula was removed and replaced with belly epidermis no longer neurally competen Completely aneural embryos were produced, in which balancers and gills but *no* nasal placode ear-vesicles, or eyes, appeared. The gills and balancers, however, were abnormally forme probably owing to lack of mesenchyme from the neural crest (a *Nachbarschaft* effect). Zwillin believes that the archenteron roof rather than the fore-brain is the inductor of the nasal placode

(2) This case led Harrison[13] to suggest the term "triple assurance". Both hind-brain (*a*) an its underlying mesoderm (*b*) possess inductive power for ear-vesicle; moreover ear-vesicle con petence (*c*) seems to be more intense in the presumptive ear-vesicle district than in other ectoderm But the inductor may well be transmitted by diffusion from the mesoderm to the hind-brai and variations of competence in different regions are not uncommon. The prime factor is therefo the head mesoderm.

(3) This was assured by O. Mangold's experiments[13], in which the whole of the endoderm w removed from a young neurula, leaving only the meso-ectodermal-neural "mantle".

(4) If the endoderm of the foregut is denied access to the skin by transplantation of a piece belly endoderm, no mouth or mouth-parts ever form.

(5) R. G. Harrison's work[1] on the development of the lateral-line sense organs was the fi in which two embryos of different species were grafted together with a problem of causal mo phology in view; its historical importance has already been referred to (p. 148). Stone's paper contain a detailed analysis of the remarkable backward migration which the post-auditory placo carries out in forming these sense-organs.

(6) The role of the mesoderm in inducing ear-vesicles is shown in a beautiful experiment Pasteels[14] where after centrifuging in the cleavage stages (see p. 221) the neural tube and notocho were almost entirely suppressed; nevertheless two well-formed ear-vesicles were induced at t anterior end of the mesoderm (see also Zwilling[2] and O. I. Schmalhausen). Ponomareva[2] fin that competence for this induction persists until the end of the late tail-bud stage.

a piece of cartilage which moves backwards and nears the skin. This is the ular tympanic cartilage, and it induces in the skin the tympanic membrane he outer ear. Fig. 174 shows an experiment of Helff[6], who transplanted the ilage to parts of the skin other than that where it normally forms, and was ays able to obtain by this means a typical tympanic membrane. Without the of cartilage no membrane will ever form. Moreover, the property of inducing npanic membrane appears to be common to many other cartilages in the body, in normal development the annular tympanic cartilage is the only one not elded by muscle, and so the power is never exercised (Helff[11]). The yellow ous part of the *lamina propria* of the tympanic membrane itself also appears lepend for its origin on the presence of the tip of the columella (Helff[7]). inductive power of the annular tympanic cartilage lasts into adult life, and e surprisingly, so does the competence of the epidermis (Helff[6]; I. Sato). Once membrane is formed, it does not regress after transplantation to the back lff[12]).

o strong is the inductive power of the annular tympanic cartilage that it can n apparently override a prior determination. Helff[8] showed that the site of the nal plicae on the head is determined rather early, but they do not appear till thyroxin period. Transplanted back to non-metamorphosed larvae the plicae ess; then if moved again over the annular tympanic cartilage, they will form npanic membrane (Helff[10]; Helff & Stark). It seems that this is only possible neteroplastic transplants; in homoioplastic ones regression occurs but tym-nic membrane competence is not retained (Helff[13]).

astly, Helff[15] has shown that the membranes induced by dead tympanic tilages are nearly as typical as those induced by living ones. A diffusible stance is therefore almost certainly involved.

he inductor of the gills is the branchial endoderm. The gill district of the oderm moves into place over it before the induction begins (Murtasi[1]). The

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But Holtfreter[19, 20] believes that anuran ventral ectoderm alone in isolation will produce ers, and so will presumptive neural plate, though for good shape and typical form the endo-al stimulus is necessary. Here, then, the inhibitory action of the individuation field may be ork (cf. p. 282).

Should this induction be interfered with, as in the centrifuge experiments of Beams & [2] or the transplantations of Burch, severe endocrine disturbances will attend the absence of nterior pituitary, including *inter alia* an absolute inhibition of metamorphosis (see p. 454), and ibsence of the normal black coloration. This is due to the maintained contraction of the nophores, not to any interference with melanin synthesis. Spontaneous cases of failure of this ction have been reported (Holt in a pig; Fawcett in a dogfish); cf. p. 454 f.n.

Cf. p. 337.

Atwell & Taft's autoplastic heterotopic grafts of presumptive anterior pituitary apparently rentiated without the stimulus from the posterior (neural) part. But the stimulus might have effective before their experiments began. Etkin & Lotkin believe that the remainder of the d exercises an inhibitory effect on the growth of the *pars intermedia*.

It has been suggested that in mammals the mammary ducts are induced by the epithelial owths around the nipple (Deakin); and the presence of accessory nipples on all parts of the (Hartman[1]; Landauer[26]) suggests a widespread ectodermal competence to a, sometimes errant, ctor.

In birds inductor phenomena are suspected in the development of the feather rudiments, so oughly studied by the Chicago school (Lillie[10]; Lillie & Juhn; Lillie & Wang; Fraps & n; Juhn & Gustavson; Juhn, Faulkner & Gustavson). It seems that the rachis can induce s and a new ventral locus, for twinning and considerable regulation can occur in feather lopment.

) See p. 319.

gradual restriction of gill-forming competence to the gill district has been the subject of particularly careful work by Ichikawa[1], which lends itself to quantitative description. As would be expected from the work of Holtfreter[5] (see p. 274), gill-forming competence is at first present all over the surface of the neurula, except on the neural plate itself. Thus Harrison[11] obtained gills from presumptive limb-bud ectoderm. Later on it gradually disappears from behind forward, concentrating finally in the gill district. This was ascertained by transplanting

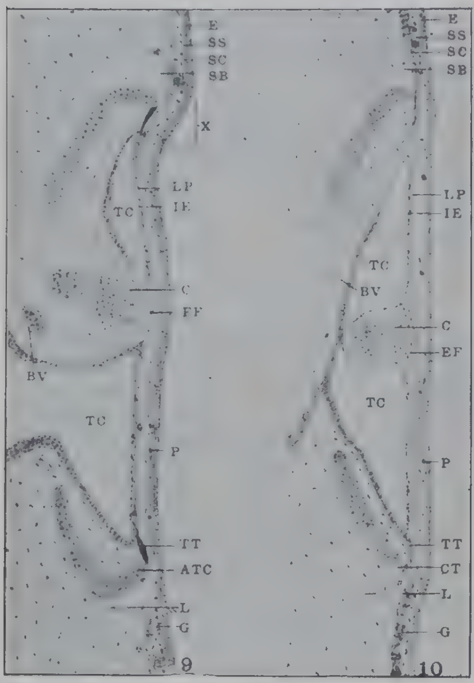

Fig. 174.

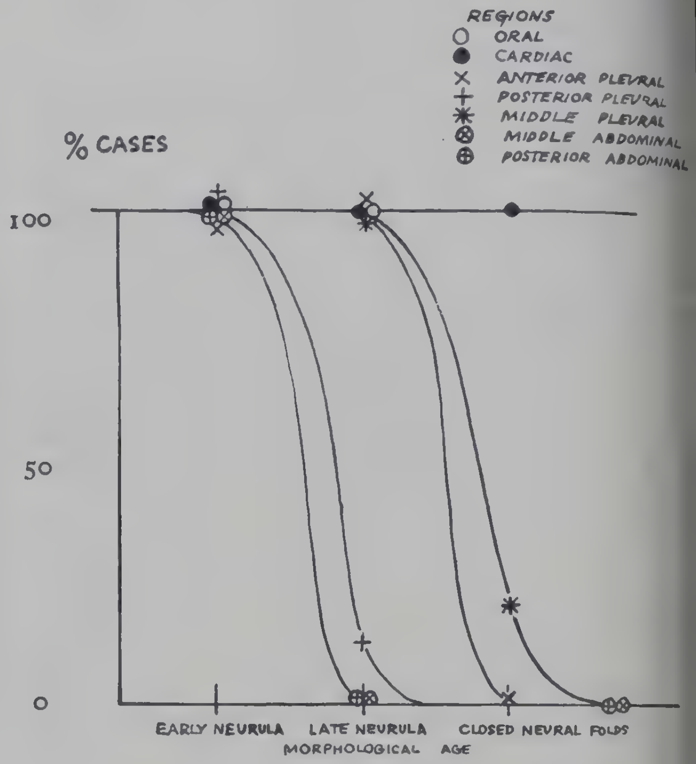

Fig. 175.

Fig. 174. Induction of the tympanic membrane by the annular tympanic cartilage. *E*, epidermis; *SS*, *stratum spongiosum*; *SC*, *stratum compactum*; *SB*, subcutaneous connective tissue; *LP*, *lamina propria*; *IE*, inner epithelial layer; *P*, pigment masses; *C*, tip of columella; *EF*, elastic fibre region of *LP*; *TT*, *tensor tympani*; *TC*, tympanic cavity; *ATC*, normal annular tympanic cartilage; *CT*, annular tympanic cartilage transplanted; *G*, skin glands; *L*, lymphocytes; *BV*, blood vessels; *X*, transition region between membrane and skin.

Fig. 175. Progressive falling off of gill competence outside the gill-district (embryos of *Triturus pyrrhogaster*).

pieces from various areas to the gill area and observing whether or not gills were formed. By plotting the percentage number of positive cases against the time (or rather, the stage of morphological development) we obtain a picture showing the decline of gill-forming competence (Fig. 175).

Like the ear-vesicle inductor, the gill inductor is not species-specific (T. H. Shen; Rotmann[1]).

The only other work of biochemical significance on second- and third-grade inductors is that of Lopaschov[7]. Having found, as we have seen (p. 298), that dead eye-cups would produce further eye-cups from isolated ectodermal balls, and that living eye-cups, placed in the blastocoele, produced only lenses, he tried the effect of placing dead nasal rudiments and ear-vesicles in the blastocoele. No

tions of any kind resulted. He concluded therefore that the substances
nsible for inducing those structures were exhausted or not further
ible.

e interplay of competences and inductors at the beginning of the self-
entiation period must be a very complicated one, but the potentialities
led by transplantation experiments probably rarely come into action in
e. Thus lens competence is present in the gill district (Spemann[5]), the gill-
ct ectoderm can act as tooth inductor (Adams), but belly ectoderm, unlike
h ectoderm, has no tooth competence (Ströer), and head ectoderm cannot
nd to the balancer (Carpenter) or the limb-bud (N. K. Arnold) inductor
. 437).

953. Limb-bud induction

e come now to the question of limb-bud induction. This problem has given
o a considerable literature of its own, which can be explored through the
ws of Mangold[6] and Balinsky[4,5] and the books of Spemann[17] and of Huxley &
eer[2]. The determination of the polarity of the limb-buds, in particular, has
ght to light facts of the greatest interest, for the different spatial axes of the
-buds are not determined at the same time. We shall, however, postpone
onsideration of this till p. 666, where it will be examined in connection with
properties of liquid crystals. For a general discussion of limb-bud deter-
ation the reader must be referred to the reviews already mentioned, since the
ect has not as yet reached the stage at which biochemical experiments have
performed. It may suffice to say that in the earlier stages of limb-bud
lopment far-reaching regulation is possible, and as many as three limbs may
roduced by dividing one bud (Tornier[1] on *Pelobates*; Lecamp on *Alytes*).
he same time the work done included one famous experiment which has
ed rather a stumbling-block, and which must be mentioned here.

Balinsky[1] has said, limb-bud induction differs from other phenomena dealt
in this section in that no obvious source of the induction is to be seen. If
-bud corresponds to lens, nothing exists corresponding to eye-cup. The
plate mesoderm, which itself takes part in the formation of the limb, would
st sight, at any rate, not be suspected.

odern work on limb-bud induction arose in connection with the induction
e cartilaginous auditory capsule by the ear-vesicle. About 1914 Filatov[1]
ed that the ear-vesicle, if transplanted to other parts of the body, would
ce an auditory capsule from mesoderm which would never normally have
ed it. After the war a younger Russian worker, Balinsky[1], tried to repeat this,
g side-plate mesoderm as the source for the auditory capsule, but found
ead to his astonishment that a supernumerary limb was produced over the
splanted ear-vesicle. This was confirmed by Filatov[2] and has since then been
ated hundreds of times with the same result.* Balinsky[3] suspected the inter-
ion of inflammation processes and tried therefore to reproduce the result by
implantation of a foreign body. In one case, and in one case only, a super-

* See e.g. Machabeli.

numerary limb was formed after the implantation of a small piece of cello Balinsky himself has never laid undue emphasis on this single result, w however, has loomed very large in the literature of experimental morphol Spemann rightly says that it deserves further investigation ([17], p. 217).

The pertinent criticism of Stone[2] that the limb-bud, after implantatio ear-vesicle, develops from skeletogenous mesenchyme inadvertently transpla with the latter, was at first only rebutted by the care with which Balinsky disse the ear-vesicle, but later Balinsky[2] obtained successful heteroplastic induct using an ear-vesicle of *Hyla* in the body-wall of *Triton* and showing that resulting limb was formed of *Triton* tissue. The criticism lost further force w the nasal grooves were found by B. Glick and Choi to be an even more powe limb inductor than the ear-vesicle, though they are situated farther away f the limb district than the ear-vesicle. Conversely, the workers of the Rus school could never get limb inductions from pronephros tissue, though th situated nearer to the limb district than is the ear-vesicle. Balinsky[6] now nasal tissue rather than ear-vesicle for preference. Eye-cups, on the other h were found by Detwiler & van Dyke to be ineffective in limb-bud induct and the pituitary rudiment by Filatov[5] to be effective.

Later interesting papers by Balinsky[5] have been devoted to the spatial and t relations of limb-bud induction. Using both ear and nose transplants he stud systematically the frequency of limb inductions at different antero-poste levels. The results are shown in Fig. 176. The frequency of induction of free lin falls off fairly continuously from the fourth to the fourteenth segment, but fr the eleventh posteriorly the frequency of inductions of pelvic girdles r steeply.* This is a beautiful illustration of the quantitative falling off of spec organ-building intensity at the "edges" of the various organ districts (cf. p. and Fig. 161). Limb induction by nose-rudiment was further studied in Balinsk counts of mitotic rate[6]; he found that the nose has a higher rate than the e vesicle, and is inclined to attach much importance to this, interpreting the wh effect in a Childian way. He has also presented valuable data on the decline competence for limb-bud induction with time, and finds that the induced lin always appear somewhat later than the normal ones, and later, too, the m caudal the segment in which they are being induced.† If nasal placode is pres as inductor, the early stages of limb formation will proceed after transplantat of side-plate mesoderm and ectoderm to the head region (Poležaiev[11]), and after their isolation *in vitro* (Poležaiev[10]).

Putting all these facts together, we may well ask what is their meaning. Th discoverers, experimental morphologists, have up till now been content c with the vague and unsatisfactory hypotheses of "gradients of metabolic rat "bio-electric potentials", etc. It can certainly do no harm to formulate m ise working hypotheses, and the biochemist, undeterred by the famous idin experiment, will first of all propose the examination of the embryo for ific substances. Only if this should fail shall we be entitled to fall back on e complex explanations. It is of course obvious that the normal inductor he limb-bud is neither the ear-vesicle nor the nasal placode, but why should

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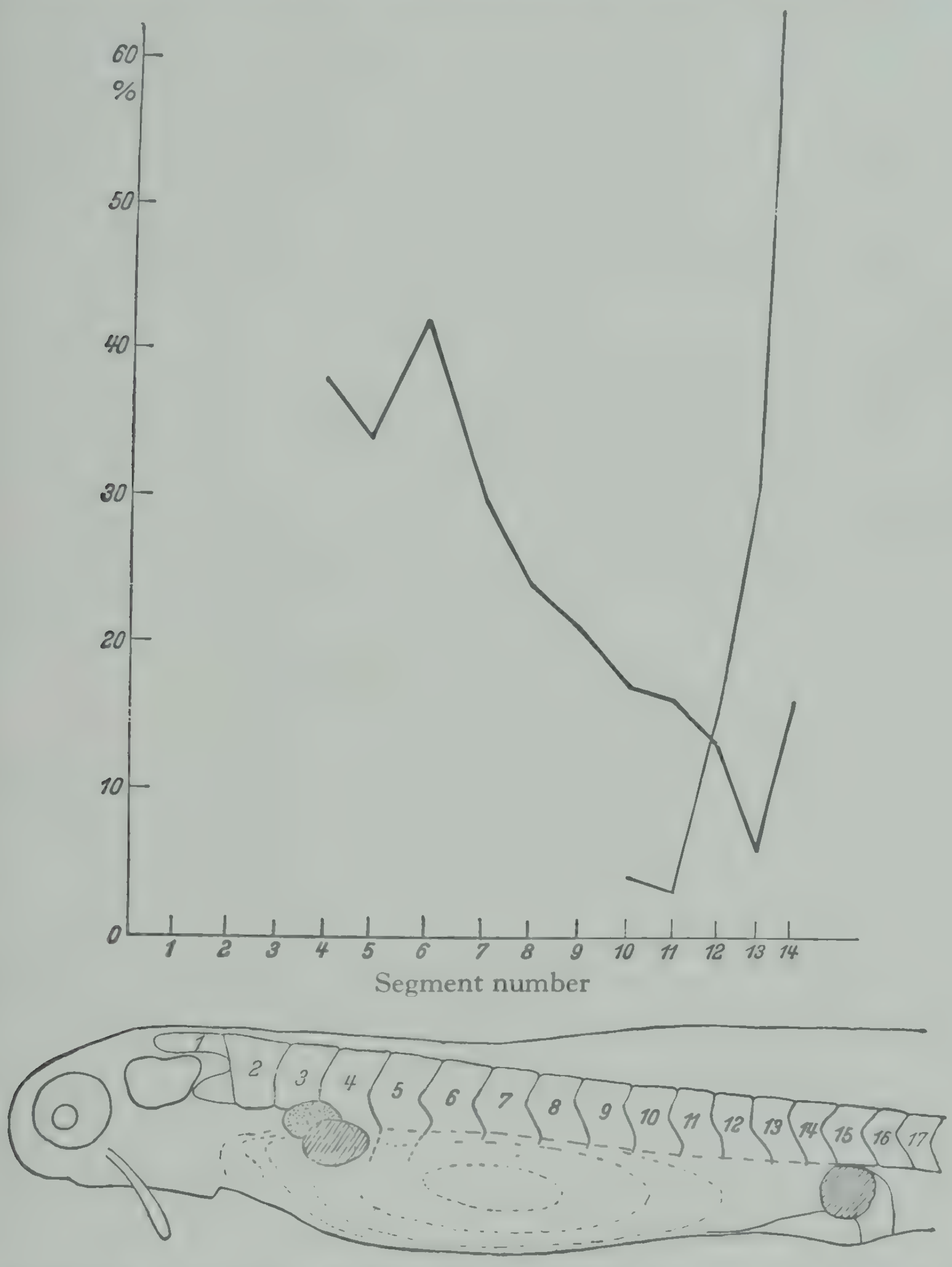
* As Balinsky[5] and Mangold[6] have pointed out, there is a phylogenetic interest in this wide of the limb district, since anciently every metamere had great developmental expression (cf. segmental appendages of polychaetes to-day). There are still species-differences among amph as regards the ease with which limbs may be induced (Balinsky[9]).

† Interesting, though puzzling, experiments on limb induction in late larval and even adult have been described by Nassonov. We shall return to this subject later on (p. 438) in connec with regeneration.



176. Frequency of induction of fore- and hind-limbs by implanted ear and nose tissue, according to the fore- and hind-limb districts. Thick line, free limbs; thin line, pelvic girdles.

se structures not contain a substance which, while not identical with that mally present at the centre of the limb-bud area in the mesoderm, would y out the same morphological stimulus? That such a substance *does* normally ear in the limb district is suggested by G. Hertwig's homoiogenetic inductions[2] hich transplanted limb-buds cause the production of new limb-buds. We e already seen (pp. 262 ff.) many cases of overlapping physiological effects caused by substances the chemical constitutions of which resemble each o in various ways. We have already seen (pp. 172 ff.) that evocators exist in m places where they seem to have nothing whatever to do, such as adult tis Had Balinsky's transplantations never been made, the ear substance would ne in nature, have had the opportunity of demonstrating one of its properties, the same may be said of oestrone, never present, as far as we know, in amphibian egg, but capable of inducing a secondary embryo if brought into con with the competent ventral ectoderm. As for the celloidin experiment, we h already seen that injuries to a tissue may liberate the masked evocator whic contains. There is nothing against, and much in favour of, the view that normal limb evocator is only liberated after a certain time in the side-p mesoderm. This is also the view of Poležaiev[6], who speaks of an "*X*-substanc Hence the isolated case of Balinsky's celloidin would correspond to the w inductions of neural plates by Okada's kaolin. To allow such experiments seduce us from following up the obvious chemical meaning of all the depend differentiations which have been unravelled in embryonic development du fifty years of the experimental morphologists' work is to render nugatory wonderful intellectual and operative effort.

The next experiments on limb-bud induction should certainly be the st of dead ear-vesicles and nasal placodes, and a study of the effects produced implanting adult tissues, alive and dead, in the presumptive limb districts.

2·3954. Opercular perforation

Before leaving the subject of limb formation for the present, we must de a brief discussion to the curious phenomenon of opercular perforation, si although it has little intrinsic morphological interest, it was the first origin of theory of "double assurance" which was referred to above (p. 293).

In late tadpole stages in anurans the gills are overgrown by a fold of skin, operculum, at the posterior edge of which there is a hole serving for the pass of water in respiration. Inside the opercular cavities the fore-limbs deve In order that they may reach the outside the operculum must be perforated; happens over the elbow, and was at first supposed to be due to mechan pressure. But in 1906 Braus[1] removed the limb-bud at an early stage from ins the cavity, and found nevertheless (in a few cases) that the hole in the opercul appeared just the same. The perforation would occur, therefore, in the abse of mechanical pressure.

Unfortunately, it is certain that many more factors are at work in this comp situation. The histolytic products of gill degeneration may be decisive in operc perforation. In addition to this, there are numerous cutaneous glands wit the cavity, both in the skin of the limb-bud and in that of adjacent areas; th may also have a part to play. And finally, there is the general internal medi with its thyroxin effect, to be considered. We have therefore five factors at le to contend with: (1) perforation as a self-differentiation phenomenon of opercular skin, (2) mechanical pressure from the limb, (3) secretion of cutaneous glands, (4) products of the autolysing gills, (5) thyroxin in the bl circulation.

acts have accumulated as follows. Helff[3] at first placed most responsibility the autolysing gills, for if transplanted to the back, skin perforations occurred r them there. Transplanted histolysing muscle acted similarly. In later k[14], however, he found that opercular skin, transplanted to the back, showed neral, not a local, histolysis, and that skin from side or back transplanted to opercular site, histolysed and perforated there. Then Weber directed ntion to possible products of the skin glands. It is doubtful if they ld all be removed by transplantations of the limb-bud, even including the ulder girdle, to a site under the skin of the back, as was done by Blacher, sner & Voronzova, who found that the limb-bud did not perforate on the k. These workers, like Helff, obtained perforation on the back, however, if degenerating gills were placed under the skin there. But then they found t opercular skin, grafted by itself to a site on the back, also perforates in due rse; and this was controlled by transplantations from other places such as tral surface and flank; these never perforated. Finally, skin from back or e transplanted to the opercular site refused to perforate, and the limb was manently retained within the cavity (contradiction of Helff). Later Liosner oronzova[1] used larvae of different ages. Opercular skin transplanted forwards older hosts perforated sooner than that of the donor controls, though later n that of the hosts, thus showing the influence of the internal medium. nsplanted backwards to younger hosts, it perforated sooner than theirs; e no influence of internal medium would be expected. Finally, limbless foration is by no means a constant phenomenon, and its frequency varies m species to species. Injected thyroxin (in the hands of Liosner & Voronzova[2]) elerated without dissociating the processes, but an interesting shift of the ring was obtained in this way by Alphonse & Baumann using 1/50,000 roxin. The time relations were changed so that sometimes the operculum appeared before the gills had degenerated at all, and in other cases the gills generated without affecting the operculum. Alphonse & Baumann noted that operculum is supplied with blood from the fifth and sixth branchial arches, l put forward the view that the real cause is the atrophy of the blood-supply ught about by thyroxin.

rom this account it will be quite evident that the complexity of the causative ors is in the case of the opercular perforation as yet too great to have been ntangled by experiment. That morphogenetic stimuli are involved we cannot bt, but we need to know much more about the latent periods of such stimuli the properties of the competence involved. Only when it has been clearly ved that at some stage in the process two or more leading factors have simulous causal responsibility shall we be entitled to have recourse to the notion 'double assurance".

·396. Second-grade organisers and caste differentiation in social animals

All the inductors so far considered operate when their due time comes in every rmal ontogeny. If, however, certain second-, third- or fourth-grade inductors re to operate only in certain individuals, differences of a social order would be up, and it may be that something very like this happens in caste differentiation ong social insects. The ant colony itself may be said to have an ontogenesis its own (Wheeler[1], p. 438). Moreover, since such substances would seem to er the developing organisms mainly by the mouth, the line between inductors and vitamins becomes as thin as that between inductors and hormones. T "royal jelly" on which the developing queen bee is nourished* (analyses Melampy & Jones; Townsend & Lucas) has been thought to be rich in vitami (claimed by Hill & Burdett; denied by Mason & Melampy and by Evans, G. Emerson & Eckert) and in gonadotrophic hormone (claimed by Heyl; denied Melampy & Stanley). Whatever the properties of the royal jelly may be, rec investigations (G. B. Castle; Light, Hartman & O. H. Emerson; reviewed A. E. Emerson[2]) have led to the remarkable discovery that alcohol or et extracts of termite queens or soldiers, fed to the population of the hive colony, are just as effective as the living queens or soldiers themselves in p venting the appearance of further queens or soldiers. Such inhibitory substan could be called worker-inductors, or they might be regarded as inhibitors of queen- and soldier-inductors which in ordinary circumstances would regener these types in the competent community. If there are really such "so hormones", and the evidence for them is impressive, they would readily spr through the hive since the colonial insects are constantly licking each oth secretions, a process which Wheeler ([2], p. 230) called *Trophallaxis*.

2·397. Sex determination

The subject of sex determination perhaps hardly falls under the head morphogenesis, since compared with the main construction of the body its effe are relatively trivial, however important for the individual and the species th may be. But as the early development of the gonad undoubtedly invol reactions of inductor type, a brief account of it is necessary. Indeed it may tu out that the inductor substances here involved will be the first second-gra organisers to which we can assign a definite chemical constitution.

That the sex of vertebrates is genetically conditioned is now a commonpla Reference to any standard account, such as that of Waddington[22], will show th since 1902, when McClung discovered the sex chromosomes, it has been unde stood that the two sexes carry different factors whereby maleness or femalen is determined. But it is only more recently that the mechanism of action of th chromosomes has been discovered. As we learn from reviews, such as those Willier[1] and Witschi[8], the prospective germ-cells arise (taking the amphibian representative) from the dorsal wall of the gut endoderm and become embedd in the splanchnic mesoderm as it moves in from the two sides to form the dor mesentery.[†] The median plate so formed then splits longitudinally into t bundles which move outwards. The next step is the separation of chains of ce from the blastema of the mesonephros, which migrate into the gonad to for

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[*] Since it is apparently a salivary secretion, it recalls the "pigeon milk" mentioned on p. Convergent evolution? The caste development, moreover, strongly resembles a determinat process (Wheeler[2], p. 199) in that if larvae are taken from worker cells after different lengths time and fed on royal jelly, the resulting queen differentiation is progressively more and m imperfect. It is interesting that nature, too, performs experiments which interfere with nor caste differentiation, as in the well-known parasitism of ants by *Lomechusa* and other staphyli beetles, in which forms intermediate between the proper castes occur in large numbers.

[†] In vertebrates other than amphibia the germ-cells may have more migration to do than t (see the reviews of Heys and Danchakova[2]).

entral core, or medulla. Thus the endodermal germ-cells are associated with sorts of mesoderm, a cortex with female potentialities and a medulla with le potentialities. As has been known since the pioneer work of Laulanié, their depends upon the genetic constitution of the animal. *In a genetic female the ex develops* at the expense of the medulla and induces the formation of eggs; *genetic male the medulla develops* at the expense of the cortex and induces formation of spermatozoa. No other mesoderm has competence to form either tex or medulla. In addition to the gonads there are of course also the sex duct tems.* The Wolffian duct,† which has male potentialities, arises as the nephric or segmental duct on each side of the body; the Mullerian duct,‡ h female potentialities, originates generally quite independently from the lomic epithelium. Out of these all the later sex ducts are formed, and although h appear in every organism only one system persists and develops, according which hormone is poured into the blood stream, the other degenerating into tigial structures. Such vestigial structures are (in man) the hydatids of rgagni (cf. p. 263), which are the remains of the Mullerian system in the male, the Epoöphoron and the canals of Gärtner, which are the remains of the lffian in the female.§ To complete the picture, the secondary sex characters ist be mentioned. Pigmentation, spurs, combs, wattles, external genitalia, essory sex glands, etc. are all, as has been known for many years, under the ntrol of the sex-hormones.‖

It may thus be seen that in sex differentiation there is a continuous succession inductors, first the nuclear inductors (cf. p. 408), which determine whether tex or medulla shall develop, next the inductors of the cortex and medulla mselves, which settle the fate of the germ-cells and the gonadal tissue, and tly the sex-hormones produced by the formed testis or ovary, which control development of the sex duct systems and the secondary sexual characters. out the first of these nothing is known. About the last we know a great deal, ie of the chemistry of which has already been discussed (p. 252). What can said of the inductors of the cortex and medulla?

hat the fate of the germ-cells depends on which part of the gonad gets the er hand, cortex or medulla, was proved by the transplantation experiments Humphrey. At a stage earlier than that of any gonadal differentiation, the flank soderm on one side was exchanged orthotopically between different embryos. er completing development it was found that the sex of the gonad on the trans-

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f these a very convenient introductory account will be found in the little book of Wiesner[2].

amed after C. F. Wolff (1738–1794), whose *Theoria Generationis* appeared in 1759.

amed after Johannes Muller (1801–1858), whose *Bildungsgeschichte d. Genitalien* appeared 830.

he suppressed system may sometimes manifest itself abnormally in later life, as when the öphoron gives rise to parovarial cysts. The contents of these, which may attain a quantity h as 6 litres, seems to be an ultrafiltrate from the blood (Dierks & Becker).

hick embryos of both sexes develop combs and spurs, but in females these soon degenerate uvier; Danchakova & Kinderis[1]). This would be expected, since in birds the homogametic is the male (see below). Precocious development of these characters may be obtained by cting male hormone (Domm & van Dyke). This is an instance of competence normally ceding the appearance of the inductor.

planted side was often different from that on the normal side, so that on t transplanted side the germ-cells had come to lie in a gonad with a differe genetical determination from that on the normal side. They may therefore devel at variance with their genetic constitution, according to the induction to whi they are subject.

That the mutual influence of cortex and medulla is carried out by substanc has been proved largely by parabiosis experiments, the literature of which h been ably reviewed by Witschi[4]. When two amphibian larvae of opposite sex are grafted together side by side in early stages, they live a long time and procee far towards sexual maturity. But the gonads antagonise one another. In toad it is true, the effects are small, presumably because the substances are not ve diffusible.* But in frogs, the male medulla will suppress the cortex of the ova nearest to it, though the diffusibility is insufficient to affect the further ovar Evidently the substances travel through the tissues rather than through t blood stream. Moreover, if the distance is too great, as it is when the anima are grafted in chain position rather than side by side, no effects occur. In urodel however, the substances are more diffusible still and both gonads of the fema partner are affected, their development being suppressed rather than mascu linised. This male dominance may, however, be reversed if species of differe sizes are used (Burns[1]), and a male of small species is united with a female large species; in this case the ovaries will suppress the testes, showing that the is a quantitative factor in the balance.

The "struggle" between the cortex and the medulla may be affected in vario ways. Thus high temperature favours the medulla at the expense of the cort (Witschi[2]),† and we have already seen (p. 255) that over-ripeness of the eggs h a profound effect in the same direction.

There has been much discussion regarding the nature of the two factors, whi have been called "cortexin" and "medullarin". They are certainly not specie specific in amphibia. Their low diffusibility suggests that they are not identic with the sex-hormones as we know them in later development (Witschi[6]). But the last few years it has become possible to apply high concentrations of crystalli sex-hormones to embryos still in the indifferent stage of development,‡ and t reversals which have been obtained have been very complete. Cortexin and lullarin may perhaps be protein complexes of oestradiol and testosterone pp. 206, 252, 316). It is at any rate clear from the work of Witschi[8] and his ol that the effects of the hormones (applied by injection or absorption) and inductors (applied by parabiosis) may differ. Thus salamander tadpoles treated oestrogens show feminisation, but in parabiosis the male partner sup-sses ovarian development. And frog tadpoles treated with androgens show culinisation, but in parabiosis the partners have no effect on each r.

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* That this is so appears also from the fact that the anterior third of the gonad in toads ne develops any medulla and hence always becomes of ovarian character. This is the long-kno Bidder's Organ. The Esthonian F. H. Bidder described this curious phenomenon in 1846. the rest of the gonad is removed in later life, this vestigial ovary will awaken and produce fertilisa eggs (Guyénot & Ponse[1]). Another remarkable occurrence is the presence of "testicular eggs" toad and frog testes which have undergone some degeneration, due, for example, to tra plantation (Welti). This is to be explained by persistence of the cortical inductor in so suppressed form.

† The effects of temperature on these inductors are very remarkable. The involuted access sex organs of castrated male mice may be restored to normal by grafting an *ovary* into the (R. T. Hill; Hill & Strong). The ovary gives off a little androgen, enough to effect the restorati But implantation into the (warmer) abdomen will not do. Thus low temperature (as of ear scrotum) favours androgen production; just contrary to what is found in amphibia.

‡ Earlier investigators (e.g. Minoura; Greenwood; Kemp; Dennis; Willier[4]; and Willier & Y were unable to obtain sex reversal in the chick using chorio-allantoic grafts of gonads. P sumably the amounts of sex-hormone so introduced are insufficient to perform the necess gonadal inductions.

mong the most successful investigators of these sex reversals have been chakova and her Lithuanian collaborators; E. Wolff and the Strasbourg p; and Willier *et al.* in Chicago. It has become clear, however, that the effects e hormones differ a great deal according to the class of vertebrates studied. s can be seen from Table 23. The full feminisation of genetic male bird ryos was accomplished by Danchakova & Massavicius by introducing oestrone olved in oil into the allantoic cavity on the 4th day of incubation.* But when osterone propionate was similarly introduced by Danchakova[3], the embryos ld not survive it. Part of the mechanism of this lethal action seemed to be precocious transformation of the mesonephros into epididymis, with a sequent fatal oedema following on the suppression of normal excretory ction. Conversely, Danchakova[5] was able to produce full masculinisation of mammalian embryo (guinea-pig) by introducing testosterone propionate in oil-drop into the amniotic cavity very early in gestation.† This provided a ect experimental imitation of the classical "freemartin" effect, first described F. R. Lillie[6] in 1916. When the foetal membranes of male and female calf ryos become united *in utero* so that the circulations are continuous, the ale partner becomes greatly modified in the male direction, forming an rsex with testes and male ducts. This can occur also in pigs (W. Hughes), does not in certain mammals such as the peludo (*Dasypus villosus*) where ochorial twinning with vascular anastomoses is a regular thing (Fernandez). e freemartin the stimulating substance may, of course, be medullarin and not osterone. For the mammalian embryo, oestrone, Danchakova[4] claims, in s sufficient to give sex reversal, is quite lethal.‡

is impossible not to correlate the fact that mammals and birds behave thus ifferently with the fact that the heterogametic sex is different in the two classes ertebrates. In mammals there is always a tendency to revert to female acters, and in the early stages of sex differentiation the female hormone does

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later life after hatching, such genetic males (in the absence of further injections) revert in the direction giving intersexes (Danchakova, Vaškovičuté, Jankovsky, Massavicius & Kinderis).

his may also be done, but not so surely, by injecting male hormone into the pregnant mammal work reviewed by Korenchevsky).

he lethality of the homogametic hormone is, however, regarded by other workers in this field some scepticism. In view of facts to be mentioned a few paragraphs below it is certainly cult to understand. Several investigators, of whom Raynaud is the most successful, have ned feminisation by injecting oestrone into the maternal organism, but it has to be admitted all such effects are on the sex duct systems and not on the gonads, hence less deep-seated the reversals obtained by Danchakova.

Table 23. *Summary of sex-reversal effects on embryos of different vertebrate groups*

	Basic type	Hetero-gametic sex	Presence of oestrogen in eggs and embryos	Results of treatment with crystalline sex-hormones in the earliest possible stages			
				♀ hormone		♂ hormone	
				♀ sex	♂ sex	♀ sex	♂ sex
Mammals	♀	♂	+	Semi-lethal or no effect	Semi-lethal (some feminisation)	Maximal masculinisation (the freemartin)	Mild hyper-masculinisation
Birds	♂	♀	–?	No effect or hyper-feminisation	Maximal feminisation	Semi-lethal (some masculinisation)	Semi-lethal or feminisation if androsterone used
Reptiles	♂	♀		Hyper-feminisation	Feminisation	—	—
Amphibia	♀ but variable	Urodeles ♀ Frogs ♂ Toads ♀	+	No effect	Maximal feminisation	Maximal masculinisation	No effect
Teleostean fishes			±	—	Feminisation	Masculinisation	—

N.B. (1) The basic type indicates that sex away from which reversals occur most easily.

(2) When the heterogametic sex is male, half of the sperm have only one *X*-chromosome; when it is female, half of the eggs have only one *X*-chromosome. In amphibia, where both sexes have all the chromosomes, there is an *XY* pair.

REFERENCES

General. Witschi[3,4,8]; Burns[4]; Spratt & Willier

Mammals. Effects of ♀ hormone; Danchakova[4]; Raynaud. Effects of ♂ hormone; Danchakova[5]; Korenchevsky; Greene, Burrill & Ivy.

Birds. Effects of ♀ hormone; Danchakova & Massavicius; Wehefritz & Gierhake; Willier, Gallagher & Koch; Wolff & Ginglinger; Gaarenstroom; van Oordt & Rinkel. Effects of ♂ hormone; Danchakova[3]; Willier, Gallagher & Koch; Kozelka & Gallagher; Gaarenstroom.

Reptiles. Effects of ♀ and ♂ hormones; Danchakova[7]; Danchakova & Kinderis[2].

Amphibia. Effects of ♀ hormone; Burns[2]; Gallien; Foote & Witschi. Effects of ♂ hormone; Burns; Gallien[3]; Foote & Witschi; Uchida.

Fishes. Effects of ♀ and ♂ hormones; Padoa[2]; Witschi & Crown; Berkowitz.

seem to be needed (hence the prepotence of the male in freemartins). But irds there is always a tendency to revert to male characters, and in the early es of sex differentiation the male hormone does not seem to be needed. Were e respective hormones present normally in the early stages, they could hardly o toxic when artificially introduced. The reptiles in this respect would be ected to behave like the birds, and this was established experimentally by chakova[7] and Danchakova & Kinderis[2]. In amphibia, the basic type seems to ariable. It has long been known that in some species and races of amphibia ndividuals develop as males and in others all as females until metamorphosis, n equilibrium is attained by 50 % spontaneous sex reversals. This is known flügerian hermaphroditism. Quite often these reversals fail to go to comion, giving adult amphibian intersexes (T. H. Ch'êng). However, in amphibia, plete sex reversal in both directions has been obtained by injection of talline sex-hormones into the young tadpole (Burns[2,3]; Gallien; Foote & schi).

rior to the discovery of these facts, Wiesner[1] had already advanced the view only one of the hormones is an active agent in development, that of the rogametic sex, male in mammals, female in birds. Differentiation of the ans of the homogametic sex would thus proceed without hormonal stimulation, ough they would retain the competence for reacting to the opposite hormone. s would agree well enough with a lethality of the homogametic hormone, is open to a number of criticisms (Raynaud; Zuckerman & Groome).

n order to clear up these difficulties and confusions the data on the presence of -hormones in embryo, placenta, etc. will have to be re-examined, and a great l more direct biochemical work done. As we have already seen (p. 88), the nmalian placenta, both of male and female foetuses, contain much oestrone; there is every likelihood that it actually secretes it (E. Allen; Allen, Pratt & sy; Brouha & Simonnet; Morrell, McHenry & Powers; Parkes & Bellerby). amniotic fluid of cows slaughtered during pregnancy has been used as a ce of commercial oestrone preparations (cf. Loewe[1]). The permeability of the enta to oestrone was at first disputed (lit. in Zuckerman & G. van Wagenen), there is now little doubt of it, since Skowron & Skarżyński showed quanively that the oestrogen of the foetal tissues rises when large doses are given ne maternal organism, and Soule finds that oestrone is always at the same l in maternal and foetal bloods. According to Cole, Hart, Lyons & Catchpole Parker & Tenney, it occurs in all foetal tissues, male or female, after a certain e, and even largely in the testes of the foetal horse.*

n the other hand, the placenta (of man at any rate) also contains androgenic stances (Goecke; Goecke, Wirz & Daners; Greene, Burrill & Ivy), though y if the embryo is female. This apparently paradoxical result was confirmed fraternal twins of opposite sexes. It seems, therefore, that we must envisage lance between oestrogen and androgen in the embryonic adnexa.

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here is a curious observation of Meyerhof & Zironi that mammalian embryo extract containing estrogens, inhibits male gland activity if injected into the mammal.

About organisms other than mammals still less is known. Fellner's early cla to have found much oestrogen in the hen's egg was decisively disproved (Loew Allen, Whitsett, Hardy & Kneibert; Doisy, Ralls, Allen & Johnston), but th may be a trace, for Riboulleau now states that the undeveloped hen's egg conta 2γ/gm. oestrone, and indirect evidence of its presence is offered by Altman Hutt. During development oestrogenic hormone is certainly synthesised (Sero Montezemolo & Balboni), though according to Serono & Montezemolo it decli in amount after reaching a maximum of 10 rat units per egg on the 11th day incubation. We do not know whether the synthesis occurs only in eggs contain female embryos.

In amphibian eggs oestrogen has been found in well-estimatable quanti (Loewe, Lange & Käer), and its presence in teleostean eggs has been claim (Sereni, Ashbel & Rabinowitz) and denied (Weisman, Coates & Moses).

It seems certain, then, that as far as mammals are concerned, development both sexes takes place in a medium comparatively rich in female hormo though this is probably not true of birds.* How, therefore, it may be asked any male development ever possible? Moreover, if the homogametic horm is toxic to embryos, how is any female development ever possible? Th questions have hardly yet received a final answer. It may be that oestrogens a androgens in or around foetal tissues are combined with protein and so ina vated. After making this suggestion, I found that the binding of sex hormones proteins in normally-occurring inactive complexes has actually been dem strated (Mühlbock; Dingemanse & Mühlbock). Or it may be that althou oestrone is normally present, female differentiation does not need it, while m differentiation has involved the suppression of the duct system which posses female competence at an early stage, so that there is nothing left for the oestro to act upon. At the same time, the female systems must retain male competen as is shown by spontaneous and experimental freemartins. Nevertheless we m still suppose that in the earliest stages of mammalian female development, wh the cortex of the gonad is overcoming the medulla, oestrogen is there at wo since in birds oestrone (when injected sufficiently early) can act as cortexin.

Arising out of the foregoing discussion there are a number of interesting poi If cortexin and medullarin are really identical with oestradiol and testostero or at any rate very similar in constitution, the induction of a female gonad fr endodermal germ-cells and mesodermal medulla will constitute a case of homo genetic induction (see p. 154), as Danchakova[4] has pointed out. For an oestroge sterol will be stimulating the formation of cells which will later produce m oestrogenic sterol. Obviously this holds conversely for "medullarin".

One of the most acceptable arguments which may be brought forward agai the identity of cortexin and medullarin with the sex-hormones is that in parabiosis experiments there is so much suppression of gonadal differentiat of the opposite sex, and relatively less reversal of direction of developme though this does occur, especially in frogs. Witschi[7] has therefore been led tulate two sets of substances, inhibitive as well as stimulative.* The former ıld be more diffusible than the latter. But it remains to be seen whether all effects cannot be obtained with sex-hormones of different constitutions.

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* A differential embryonic mortality as the result of this, bearing most heavily on male chi has been reported by Landauer & Landauer, but denied by Byerly & Jull[2].

Among the experiments on the action of hormones, not a few paradoxical ects have been obtained. Of these perhaps the most peculiar is that of Padoa[1], o on administering oestrone to a type of amphibian which normally develops 0 % female up to metamorphosis (*Rana esculenta*) obtained a 100 % transmation of the tadpoles into males. Gallien on *Rana temporaria* and Witschi rown on *Rana pipiens* were unable to reproduce this result, but in view of the at differences between amphibian species this cannot be taken as a lack of nfirmation. They obtained results more in agreement with theory, the male mone masculinising the tadpoles and the female one feminising them. Wolff[2] then showed that androsterone has a masculinising effect on female ck embryos and a feminising effect on male ones, and though at first doubt s thrown on this finding, it was later confirmed for androsterone and transnydroandrosterone (Willier[3]; Willier, Rawles & Koch) and for methyl-17-lrostanol-17-one-3 (Wolff[4]; Wolff & Wolff[1]). Testosterone acetate behaves nilarly but not so strongly (Wolff & Wolff[2]). It has, however, already been ntioned that substances are known with both masculinising and feminising operties (see p. 261). Furthermore, in later life, testosterone was found by nchakova[8] to have feminising effects on the guinea-pig, and it is recognised in nical practice as possessing truly bisexual properties (Korenchevsky & Hall).

The action of the pituitary has been excluded in these sex-reversal effects. ter its complete destruction by localised irradiation (Wolff & Kantor) or its irpation (Fugo) the gonads show no deviations from normal development olff & Stoll; Fugo & Witschi), and sex reversal is still quite possible (Wolff[3]). the other hand, it seems that towards the end of incubation the gonads can pond to injected gonadotrophic hormone, if the conclusions of Domm & nnis are correct. Daineko[2] gives data on the appearance and accumulation of gonadotrophic hormone in the pituitary.

n sum, it must be admitted that the question of sex differentiation in the ner vertebrates is exceedingly complex, and will not be fully resolved until know much more than at present about the distribution of the sex-hormones the competence to react to them.

The invertebrates as well as the vertebrates have been the subject of an immense ount of research on sex determination. Here Goldschmidt's work[4] is classical. the gypsy-moth *Lymantria* gynandromorphs and intersexes are common. The mer do not throw much light on sex differentiation, for they are really sexual maerae, in which one part of the body is of one sex, and another of the other. ey arise from abnormalities in nuclear divisions whereby the female-determining *X*-chromosomes get lost, and they may consist of a complex mosaic. t intersexes are animals in which all the organs begin their development as if

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Vitschi ([1], p. 482) analogises this inhibition with the inactivation of the primary evocator in all ons of the gastrula other than the dorsal lip of the blastopore (see p. 174). But it resembles more ely certain inhibitive effects of individuation fields (see p. 282).

the animal was a male, and later switch over to complete it as if the animal we a female, or *vice versa*. This is analogous to the Pflügerian hermaphroditism some amphibia, already mentioned, except that conditions in the moths are mo flexible. The sex factors of the nucleus, in Goldschmidt's view, produce ma and female hormones, and development follows whichever of these is produc in excess. Hence, according to the speeds of the metabolic reactions produci the substances, the intersex switch-over point will occur at various times. T same considerations apply to the development of other insects, such as *Drosoph* (Bridges[2]). Unfortunately, nothing whatever is known of the nature of t substances in question. Attempts to influence the sex differentiation of insec by the mammalian sex-hormones (e.g. Danchakova & Vaškovičuté) have not far been successful, though the presence of oestrogen in adult insects has be established (Loewe, Raudenbusch, Voss & van Heurn).

No discussion on sex determination would be complete without a mention the remarkable case of the gephyrean worm, *Bonellia*, which has been studi by Baltzer[2]; Herbst[4], and their collaborators over a number of years. Convenie reviews of the subject are those of Baltzer[5] and Nowiński[2].

The female worm has a body about the size of a walnut and a long probosc projecting about a yard for collecting the mud on which it feeds. The male extremely small, never more than 3 mm. in length, and lives as a commensal the female's intestine and uterus. The egg develops into a sexually indifferer larva, which after a short free-swimming life begins to proceed in either the ma or the female direction. Which it does depends upon whether or not it becom attached to the proboscis of an adult female; if attachment takes place, it w become a male, if not it will become a female (Baltzer[2]). At the same time should be mentioned that a very small number of larvae do spontaneously develo into males without the help of the proboscis, but these genetic males do n exceed 1 % of the larval population. The process of transformation of the larva into the male line of development can be clearly followed during their stay c the proboscis (Glaus), to which they are stuck by an adhesive secretion whic certain larval glands produce (Loosli). Workers in this field thus speak of th number of "proboscis-hours" in an individual's history.

Clearly the sex determination in these worms is exceedingly labile; just th opposite of the strongly genetically conditioned insects. Experiments on th nature of the inductor substance in the proboscis have begun, but owing to th comparative rarity of experimental material, have not been carried very fa Baltzer showed that aqueous extracts of the proboscis are effective, and Nowińsk found that none of the active substance in proboscis tissue was lost to aceton but could be obtained by subsequent extraction with water. The substance quite stable to boiling. At the same time it seems that the larval system is rathe sensitive to a number of external factors; thus Herbst[4] was able to obtain fairl high proportions of males by low *p*H, by traces of copper, by glycerol, and b variations of ion concentration such as K′ and Mg″. Heydenreich did the sam with high CO_2-concentrations. On the other hand Mutscheller was able to sho by direct estimation of the copper content of the proboscis that it contains abou hundred times too little to allow the assumption that copper is the naturally urring masculinising factor.

t would appear, therefore, that a number of external conditions can set the elopment going in male or female direction. Perhaps it is not too rash to gest that what these conditions do is to liberate naturally occurring male or ale hormones—which may be identical with the products of the nucleus— m inactive combination. The role of the proboscis might therefore be that of plying an activator rather than an inductor itself (cf. the discussion on phibian neural induction, p. 188).

2·399. Organisers and recapitulation

In modern biology we speak of "recapitulation phenomena" rather than of recapitulation theory, since the facts of persistence of structures in ontogeny racteristic of embryos of species lower and earlier in the phylogenetic scale accepted by everyone. In the development of one of the higher animals, gans which were important and functional in its ancestors may be found ained in modified, and rudimentary or vestigial, form. The difficult question why a vestigial organ should be retained at all. Kleinenberg[2] was the first to gest that vestigial organs are retained because they are in fact indispensable paving the way during embryonic development for the appearance of later gans which play an essential part in the animal's economy. In an earlier cussion (*CE*, p. 1635) I made the idea more precise in view of the results of dern experimental embryology, and suggested that a vestigial organ is retained cause it provides a morphogenetic stimulus which the animal cannot do thout.[*]

The notochord is not a clear case of this. Still functional in the amphibian va as part of the skeleton, it has become vestigial in birds and mammals, and nce it might be suggested that it has been retained solely for its power of lucing the neural plate. But the induction is believed to be performed by the ole of the axial mesoderm rather than by the notochord alone.

clearer case has since then been found in the study of the development of urino-genital system. By the union of the pronephric tubules the Wolffian t is formed; it then grows backwards. The pronephros[†] is quite vestigial and ctionless. If the pronephric rudiment is removed (in amphibia) no duct arises, the mesonephros either fails to appear (Miura[1]; Waddington[17]) or is almost npletely absent (Shimasaki; O'Connor[1]).[‡] Similarly, Waddington[17] and

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thers later gave support to this view (e.g. de Beer[3]; Shumway; Filatov[9]; F. E. Lehmann[15]; ntz; H. J. Muller). Doubts, which I share, as to whether *all* cases of vestigial organs and cially vestigial behaviour patterns, can be explained in this way, have been voiced by A. E. erson[1].

irst described by Joh. Muller in amphibia (1829) and by Balfour & Sedgwick in birds (1878). amphibia the mesonephros is functional and essential to life (Howland); in birds it is functional perhaps not physiologically essential. However, the pressure of what it secretes is important the normal development of the allantois (Boyden[1]).

n this induction there is apparently a graded series of species differences in the degree of endence which the mesonephros has upon the pronephric duct. The misgivings of O'Connor[1] ing out of this are perhaps unnecessary if we remember the explanation advanced above 292) for the exactly parallel case of lens induction.

Grünwald have shown that in the chick, if the Wolffian duct is cut, mesonephr metanephros and Mullerian duct never subsequently develop. It is not necessa to speak of double assurance in cases where a trace of the latter tissue is form until we know that traces of the responsible substances do not diffuse from t pronephros.

It may thus be said that the functionless pronephros induces the mesonephr and metanephros by means of the Wolffian duct as an intermediary. It will interesting to see whether the whole range of recapitulation phenomena, whi have given rise to so much bewilderment and so many pages of speculation, w turn out to be intelligible in terms of morphogenetic stimuli, and so ultimate of biochemistry.

The wider questions which arise at this point, of the relations between organis phenomena and phylogeny, especially as regards homologies, cannot be de with here. De Beer (in a book[2] and essay[3]) brings evidence to show that (1) characte controlled by identical genes need not be homologous, (2) homologous characte need not be controlled by identical genes, (3) structures can owe their origin different organisers without forfeiting their homology, and (4) homologo structures do not necessarily arise from the same situations in the gastrula, ar perhaps not even from the same germ-layers. Bertalanffy[4], on the other han seems to maintain the converse of (3).

Another way of expressing the view here put forward about recapitulati phenomena would be to say that the retained, vestigial, or transitory, structure are examples of "correlated variation". Often discussed by Charles Darw (*Origin of Species*, p. 8; *Variation of Animals and Plants*, pp. 311 ff.), the occurren of characters, whose inward relation we do not as yet understand, has long be taken notice of. Some instances of this, wrote Darwin, are quite whimsical, su as the deafness of white cats with blue eyes. And Dollo spoke of the usele characters fixed through long periods of evolutionary change in the mari turtles, because they were "corrélatifs d'une structure indispensable".

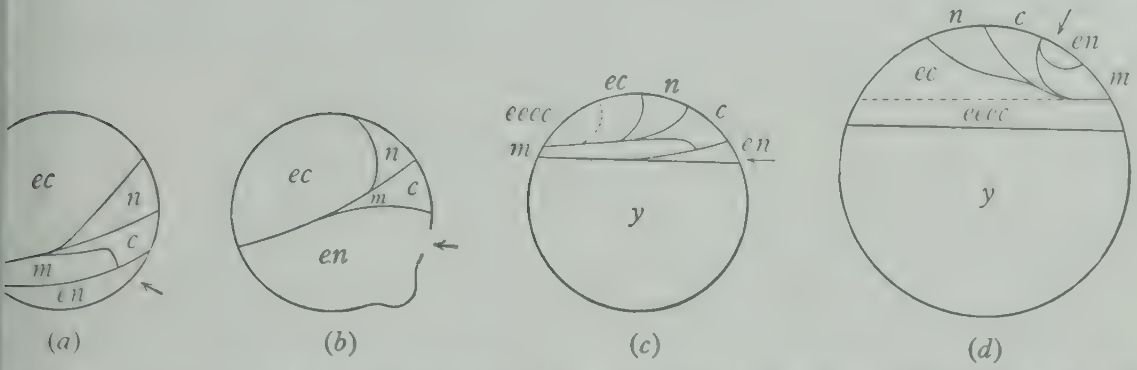
2·4. Organisers and fate-maps in Prochordates, Fishes, Sauropsi and Mammals

Until recently it was difficult to distinguish anything in common between t different modes of development among the chordates. The strictly mos development of the ascidians seemed to be in sharp contradiction with the reg lative development of amphibia, and the early rearrangements of presumpti parts seemed impossible to reconcile in the flat blastodermal system of bir with the spherical system of amphibia and fishes. Largely owing to the synthe work of Dalcq[7]; Pasteels[15] and Peter[3], however,* we are now able to see that the are gradual relationships between all these different groups. Although relative little biochemical work has been done on organiser phenomena in groups oth than amphibia, we shall give a brief description of the happenings in these oth types.

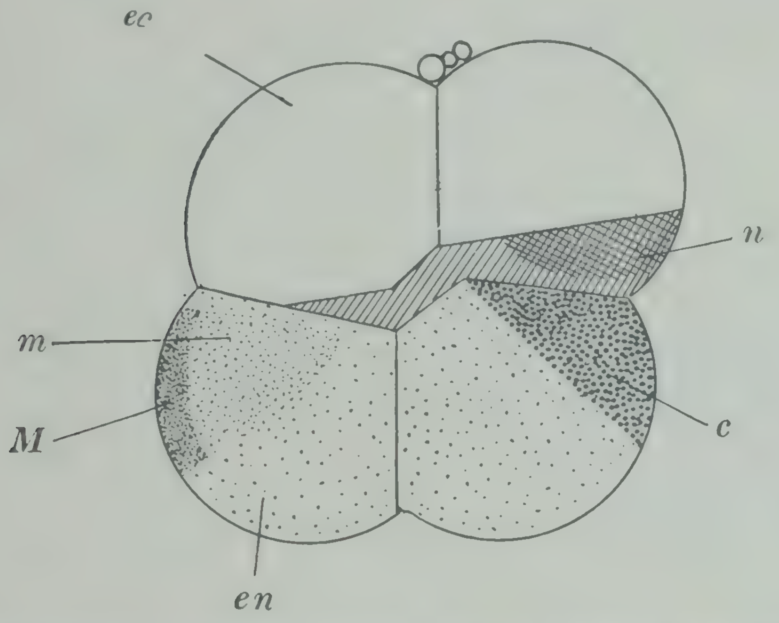
* Between the schools of Dalcq and of Peter there are still certain differences of opinion.

41. A comparison of fate-maps

rst of all, let us look again at the map of the presumptive parts of an amphibian ryo at the beginning of gastrulation. Fig. 177 (*a*) shows the fate-map of an an, the toad *Discoglossus pinctus* (Pasteels[7]); it closely resembles the map n already in Fig. 57 A for the newt, derived from the classical work of Vogt,



177. A comparison of fate-maps (lateral). (*a*) Fate-map of the toad (*Discoglossus*); (*b*) fate-map of the lamprey (*Lampetra*); (*c*) fate-map of a teleostean fish; (*d*) fate-map of a sauropsid. *n*, Presumptive neural area; *c*, presumptive notochord; *m*, presumptive mesoderm; *en*, presumptive endoderm; *ec*, presumptive ectoderm; *eeec*, extra-embryonic ectoderm; *y*, yolk. The arrows indicate the position of the blastopore.



ig. 178. Fate-map of the ascidian *Ascidiella* (lateral). Lettering as in preceding figure. *M*, Myoplasm (see p. 131).

that the presumptive neural area is smaller. If now we compare this with ap for the ascidian *Ascidiella scabra* (Fig. 178), drawn by Vandebroek[2] in eight-cell stage (cit. in Dalcq[7], p. 37), we see a considerable resemblance. presumptive neural area can be seen just above the presumptive notochord , and the rest of the embryo's surface is divided between presumptive derm and endoderm. "Who would have imagined", wrote Dalcq, "that n Conklin described the famous pigmented crescents of *Styela* and *Ciona*,

nature then revealed to him the essential features of the germinal organisat
in chordates?" These pigmented plasms have already been discussed (p. 1
No vital staining experiments have as yet been made on *Amphioxus*, but Da
believes that its arrangement of presumptive areas would be much the same.

It will be noticed that whereas in the amphibian fate-map there is a "me
dermal girdle" stretching right round the embryo and dividing presumpt
ectoderm from endoderm, the simpler prochordate system has no such arran
ment. The same simpler scheme is found in the cyclostomes, where *Lampe*
fluviatilis has been studied by Weissenberg, for here also most of the embry
surface is taken up by presumptive ectoderm and endoderm, only small crescen
areas being left for presumptive neural tissue and mesoderm (Fig. 177*b*).

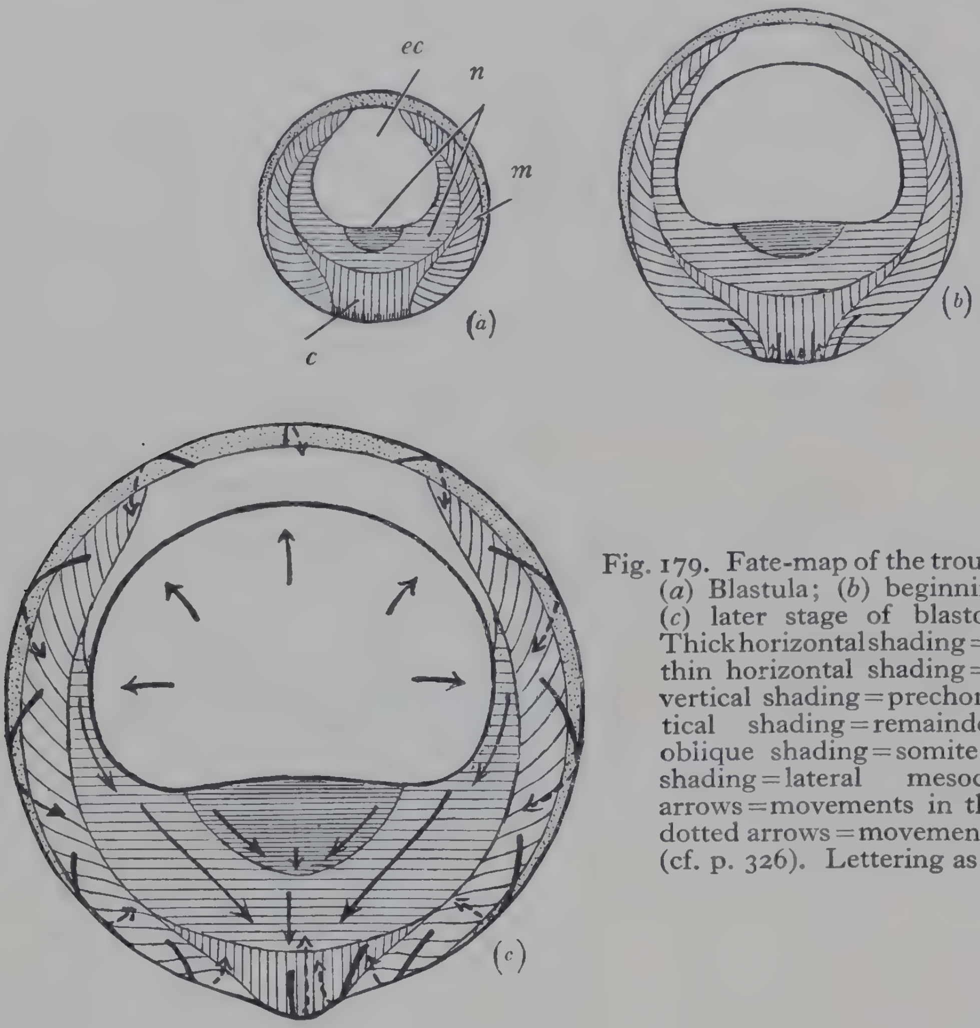


Fig. 179. Fate-map of the trout *Salmo* from abov
(*a*) Blastula; (*b*) beginning of invaginatio
(*c*) later stage of blastodermal expansio
Thick horizontal shading = presumptive brai
thin horizontal shading = neural tube; thi
vertical shading = prechordal plate; thin ve
tical shading = remainder of notochor
oblique shading = somite mesoderm; dott
shading = lateral mesoderm; continuo
arrows = movements in the superficial laye
dotted arrows = movements after invaginati
(cf. p. 326). Lettering as in Fig. 177.

When we leave the holoblastic eggs and have to deal with eggs in which t
cleavages in the yolk are few or absent, as in fishes, we find just the oppos
state of affairs, for of the area of the cap of cells which sits on top of the yo
mass, the greater part is taken up by presumptive neural tissue and presumpti
mesoderm. Gastrulation takes place by the expansion of this blastoderm (especia
its ectodermal part) so as to envelop the whole of the rest of the egg (the yo
mass), and, as the expansion proceeds, the mesoderm is tucked in underne

neural axis, first the prechordal plate, then the notochord and somites, like unrolling of a carpet, but with lateral contributions. The distribution of the s on the fate-map of the blastoderm before gastrulation, as established by the k of Pasteels[6], is shown in Figs. 179 and 177*c*, for the trout *Salmo irideus*. s was shortly afterwards confirmed Oppenheimer's work[5] on *Fundulus*. chian fishes, though they have much e yolky eggs, seem to fall into line, e diagram of Fig. 180 for the dogfish, *llium canicula*, taken from Vande- ek[1], shows. These have, however, e of the characteristics of amniote s.

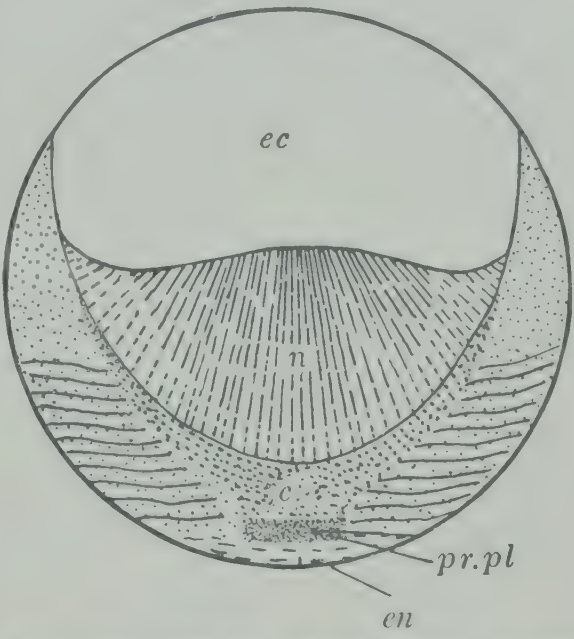


Fig. 180. Fate-map of the dogfish *Scyllium* (from above). *pr.pl.*, Prechordal plate. Lettering as in Fig. 177.

he sauropsida have been, of course, most difficult of the groups to bring a consistent series, for the gastrula- of amniotes is no longer marginal. edges of the blastoderm, sweeping wn, round, and under the yolk-mass, longer represent the edges of the stopore. The blastoderm has, in fact, d with the colossal work of absorption the nutrient materials from the yolk, ded itself into two portions, the "embryonic" and the "extra-embryonic"; while the latter is engaged in enveloping the yolk, the former has developed ew blastopore, at which invagination of the endo-mesoderm takes place, owed by the appearance of the neural axis just as in amphibia. This is wn in Fig. 181*c*, the fate-map for the tortoise *Clemmys leprosa*, established asteels[8]. The typical pattern of the amphibian dorsal region can be made out, the presumptive notochord area is sandwiched between the presumptive al area anteriorly and the presumptive endodermal area posteriorly, with the umptive somite areas on each side of it. Anterior to the presumptive neural is the embryonic ectoderm, and the whole system is surrounded on all sides the extra-embryonic ectoderm. Invagination itself takes place in a way emely similar to that of the anamniotes.

the birds the reduction of the presumptive endoderm area has gone much her than in reptiles. Fig. 181*d* shows the map of the presumptive areas posed by Pasteels[5, 9], as the result of vital staining experiments, for the chick. The ryonic area is here again surrounded by a vast area of extra-embryonic ecto- m, and the gastrulation process, which has become very complicated, differs m that in reptiles. The primitive streak, the absence of typical archenteron and henteric roof, and the movements of Hensen's node, are all new features. further details the reader must have recourse to the morphological literature.

f the endodermal area of the amphibian fate-map (Fig. 177*a*) were to be reduced rmously in size, one can see that it would appear completely enclosed by

presumptive mesoderm, as is almost the case in the birds. In this case, if
presumptive ectoderm were to expand continuously as the volume of the
expanded, it would come to surround the other, small, presumptive areas a
does in the sauropsida (Fig. 177*d*). But this does not explain the change in gastr
lation mechanism. Why should the edges of the blastoderm in meroblas
fishes preserve the significance of the blastopore lips in enveloping the yolk a
yet the corresponding edges in the amniotes not do so? Anamniote blastopo
might be called "peridiscal"; amniote ones "intradiscal". It is perhaps only

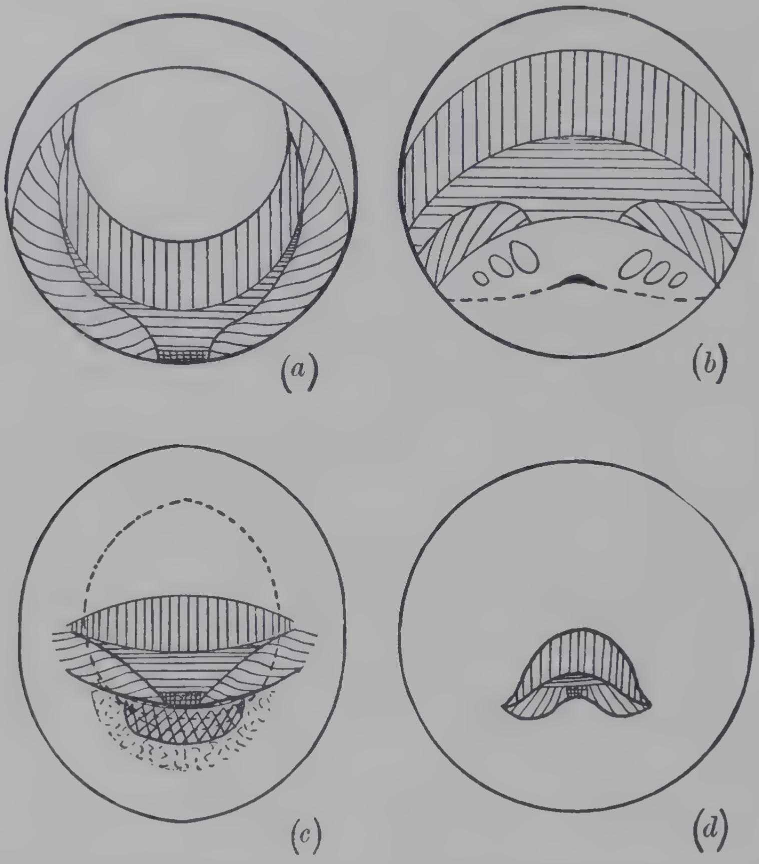


Fig. 181. A comparison of fate-maps. (*a*) Fate-map of the trout (*Salmo*); (*b*) fate-map of
toad (*Discoglossus*); (*c*) fate-map of the tortoise (*Clemmys*); (*d*) fate-map of the chick (*Gallu*
(*a*, from above; the others, facing the blastopore, *b*, from behind, *c* and *d* from above.) Vertica
shaded = *n*; horizontally shaded = *c*; obliquely shaded = *m*; vertical-horizontal cross-hatch
= prechordal endoderm; obliquely cross-hatched = *en*; dotted = lateral and extra-embryo
mesoderm. Lettering as in Fig. 177.

verbal explanation to say that with the enormous increase in the amount of stor
yolk material, there has come about a dissociation between the yolk-enclosi
and the embryo-forming functions of the blastopore. This structure has therefo
been divided into two: (*a*) the yolk-enclosing edges of the blastoderm and (*b*) t
points of invagination of the endo-mesoderm in the embryonic area. For
attempted explanation of this in terms of the Dalcq-Pasteels theory (cf. p. 22
the monograph of Dalcq[7] must be consulted. As has been mentioned (p. 14
the formation of the amnion may perhaps be referred to the characteristic ect
dermal tendency to expansion (epiboly) seen in amphibia. Enclosure of t
embryo within the amnion would necessitate the formation of the allant
diverticulum of the gut as a further consequence for respiratory needs.

ith the mammals we reach a spectacular reversal of the yolk-accumulation ess. The minute yolk-mass is vestigial. But as eutherian mammals are ably descended from ancestors having foetal membranes analogous to those he sauropsida, we should expect to find analogous processes still at work. suggested that the expansionist tendencies of the ectoderm, still preserved, unt for the formation of the trophoblastic villosities (and hence ultimately placenta), which precedes the formation of the amnion, just as does the nsion of the extra-embryonic blastoderm all over the yolk-mass in birds. o the present time no definitive fate-map has been established for a mammal, it will no doubt resemble to some extent those of the sauropsida.

·42. Determination in Prochordates

he ascidians, ever since the classical work of Chabry[1] in 1887, have been rded as undergoing typical mosaic development.* But the production of embryos from one of the two first blastomeres is no longer considered proof no regulation occurs. Dalcq[9], in numerous merogony and blastomere-lling experiments, has shown that a considerable degree of regulation is ible, although the neural rod is certainly not determined by the notochord, he neural tube is in amphibia. However, in later development, several cases nduction have been found. Whatever the origin of the ectoderm covering it, anterior endoderm induces the adhesive papillae (T. C. Tung[2]), and later on these papillae which, in some cases, give off a stimulus necessary for the rption of the tail prior to the beginning of sessile life (Holmgren; Zhinkin[2]). ilarly, the "eye" and otocyst of the ascidian tadpole are dependent upon the ence of that part of the endoderm which normally underlies the brain (Rose; debroek[2]) and the otolith upon the presence of the tail (Berrill[2]). Induction nomena exist, therefore, in ascidian development, but it is mosaic in so far as action of the primary organiser may be said to be carried out before cleavage. f the biochemistry of ascidian induction nothing is known, if we except the ted observation of Zhinkin[1] that certain dyes greatly accelerate ascidian metaphosis. Conklin[3] has the impression that during metamorphosis the tadpole es become acid, but this needs further investigation.

·43. Determination in Cyclostomes and Fishes

n cyclostomes and fishes the inductive origin of the neural axis has been ed beyond doubt by the admirable work of Bytinski-Salz[4] on the lamprey, *petra planeri*,† Oppenheimer[1,3] on the perch and minnow, *Perca fluviatilis* and *dulus heteroclitus*, and Luther[2] on the trout, *Salmo irideus*. In the case of the prey, with its holoblastic cleavage, conditions were not far removed from the phibia, and Bytinski-Salz was able to reproduce the twin embryos by inductions lmost all particulars similar to those of the classical type. But in the case of fish eggs, with their very large amount of liquid yolk, their oil-globules, and r tough "chorionic" membranes, the operational technique was much more cult. Oppenheimer, the first to succeed, was quickly followed by Luther.

See p. 99.

† Confirmed later in independent work by T. Yamada[2]. See also p. 451.

Normal teleostean development may present a beautiful picture becaus the relative transparency of the embryonic tissues and the yolk. The clas description of it is usually said to be that of H. V. Wilson[1] on *Serranus*, the sea-b but many modern descriptions are valuable, among which may be mentioned th of Price[1] on the whitefish, *Coregonus clupeiformis*, and of Solberg[1] on *Fundu* Some of the latter's photographs are here reproduced (Fig. 182). During gast lation the whole blastoderm expands to cover the yolk with a thin membrane,* invagination, as has been said, occurs only at a localised region of its edge, al a line (at right angles to the advancing edge) which lays down the future neu axis. The axis (the embryonic shield), and also the advancing edge (the germ rin are thicker than the rest of the blastoderm. Earlier investigators, restricted defect experiments, cautery, etc., had noticed that injuries to the embryo shield or the dorsal lip of the invagination were followed by great deficiencies the embryo or its complete absence (Kopsch; Sumner; Hoadley[2], etc.). Mo remarkable was the conclusion of Lereboullet as far back as 1855 (brought light by Oppenheimer[6]) as the result of his study of fish twinning, that the dor lip region had a leading part to play in the formation of the embryo: "Le bourre embryogène", he wrote, "doit donc être considéré comme un amas, une so de magasin d'éléments organisateurs, et comme le point de départ de tou formations embryonnaires, regulières ou anormales."

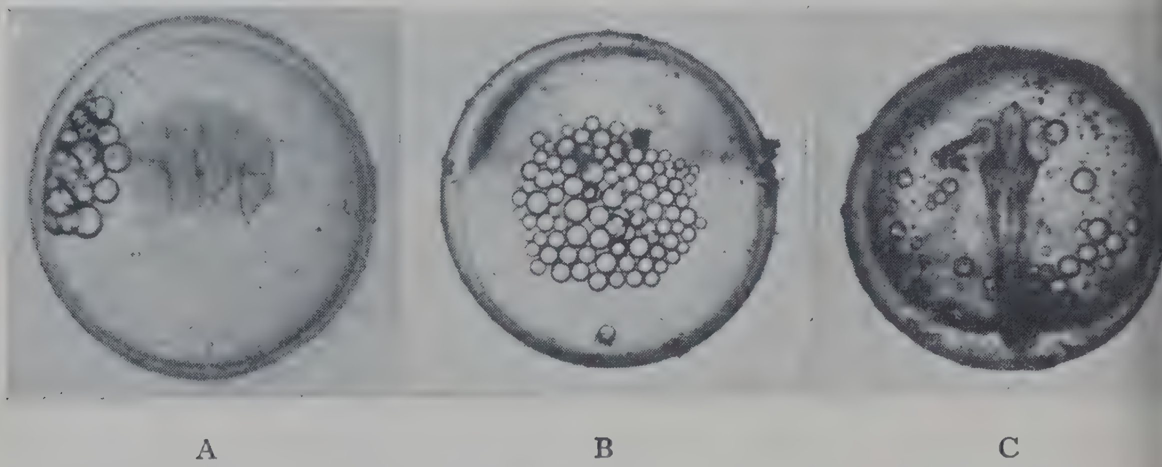


Fig. 182. Stages in the development of a typical teleostean fish (*Fundulus heteroclitus*). A, 2½ h 8-cell cleavage stage. B, 18 hr.; blastoderm growing down over the yolk. C, 42 hr.; neu axis fully formed. *A* and *C* from above, *B* from the side.

Fig. 183, taken from Luther[2], shows the way the induction experiments a performed. If the just-invaginated mesoderm is transplanted to a point und the ventral edge of the blastoderm diametrically opposite to the natural neu axis, a secondary neural axis will form just as in the amphibia. Fig. 184, tak from Oppenheimer[3], shows experimentally produced twins in later develo ment. In other experiments, Luther[4] rotated the whole of the upper layer, a obtained a twin, for the originally invaginated endo-mesoderm induced a axis in the presumptive ectoderm, and the old neural axis completed itself rther invagination on the opposite side of the egg.

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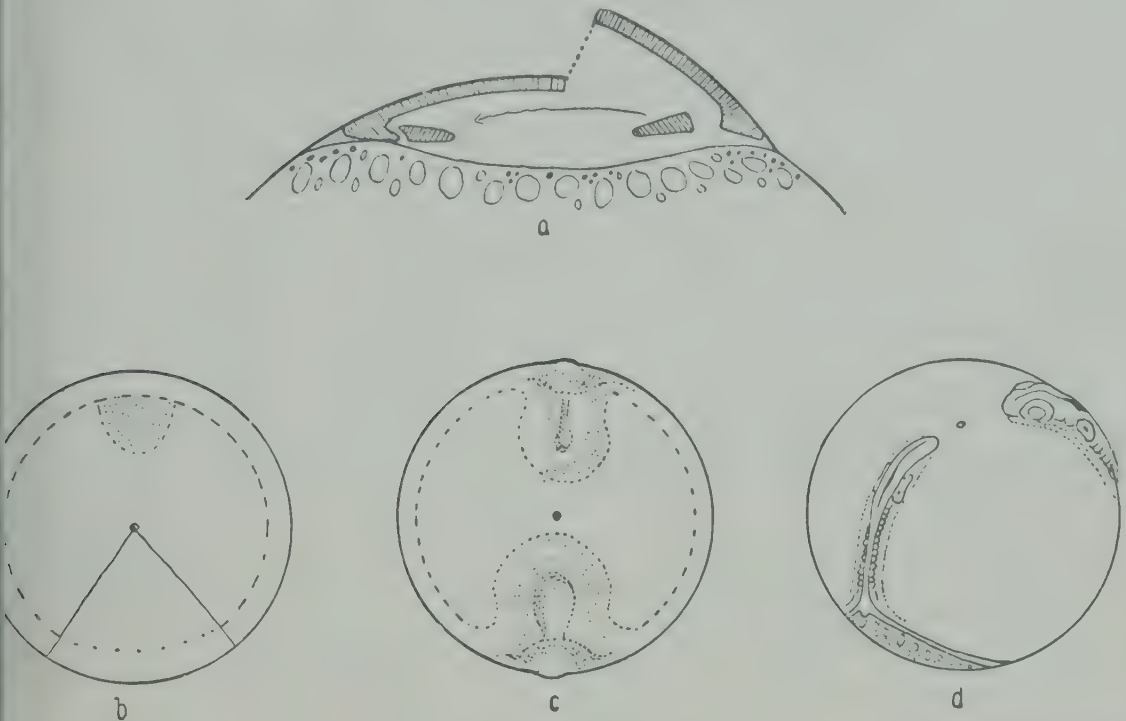
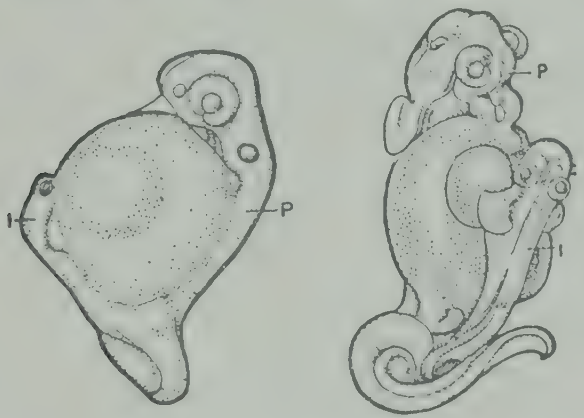
* In connection with conditions in mosaic eggs (see Section 2·2) it is interesting that teleost yolk is considerably more acid than the animal pole plasm and later the blastoderm (*p*H against 7·6, Spek[4]; cf. *CE*, p. 850). A fine picture is obtained when teleostean eggs are stai with cresyl violet; the yolk is blue, the oil-globules yellow and the blastoderm red. Trifono finds that the blastoderm becomes slightly more acid (neutral red staining experiments) development goes on, whether normally or parthenogenetically begun.



183. Method of performing induction experiments in the fish egg. (*a*) Transplantation of invaginated mesoderm (side view); (*b*) limits of operation (from above); (*c*) resulting twin embryonic axis (from above); (*d*) the same; later stage (from the side).



. 184. Induction of secondary embryos in a teleostean fish by implantation of organiser. *P*, host embryo; *I*, induced embryo.

general, events proceed much as in the amphibia. By removing a segment ciently large to include the whole of the presumptive embryonic area, Luther[5] ined anidian blastoderms where no trace of axiation was to be found. aller removals permitted various degrees of regulation,* some of which were so arkable as to give the impression that evocator can be liberated in regions outside

---

eces of blastoderm transplanted on to the yolk-sac of older larvae according to a method of Iangold[9] showed some *bedeutungsfremd* differentiation (Luther[3]), but this is probably not ortant in view of what has been said above (p. 157) regarding the neutral medium. By this od, however, the decline in competence of the extra-embryonic areas is well shown[7].

the embryonic shield where this normally does not occur. With this Oppenheim agrees. In order to test it further, Luther[5] took the extra-embryonic halves two very young gastrulae, i.e. the sectors farthest from the embryonic shi and fused them together. Contrary to expectations derived from experience w amphibia, a new organisation centre was formed, and normal embryos w produced. This is as if two *Bauchstücke*, or an ectodermal ball, should produce embryo. Later results clearly showed that the evocator in fish blastoderms is m readily liberated than it is in amphibia, for in one case (Fig. 185) where the segm of the embryonic shield had been removed, two embryos arose with their a not at right angles to the blastodermal edge, but lying actually along it (Luther

During the gastrulation process, the capacity for regulation rapidly falls and in later stages an embryonic axis can no longer make notochord and somi for itself out of adjoining blastoderm if necessary, though the regeneration ear-vesicles persists for a long time. Another example of regulation is seen blastoderms which for some unknown reason fail to envelop the yolk-mass; h

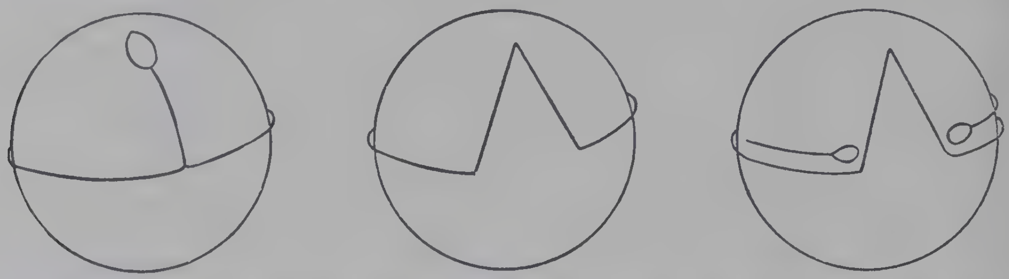


Fig. 185. Formation of twin neural axes in fish embryo along the edges of the embryonic shiel

highly differentiated but very small embryos form out of material the presumpti fates of which would have been different during normal development. As in t amphibia, presumptive epidermis and presumptive neural tissue are quite inte changeable before the action of the organiser has taken place (Luther[2]). Hea and tail-organisers are distinguishable, as in amphibia (Eakin[3]; Dragomirov).

An original experiment undertaken by Oppenheimer[2] was to make teleoste development "holoblastic" by removing embryos from their yolk at vario stages and cultivating them in Holtfreter solution. If this was done before t 32-cell stage, a variety of chaotic "hyperblastulae" were produced with litt histological differentiation. If afterwards, all kinds of histological differentiati (neural, notochordal, etc.) took place, but abnormal topographical relation possibly owing to the abnormal water metabolism caused by absence of the yo (see *CE*, p. 890), occurred. The evocator is thus not liberated when the blast derm is isolated from the yolk before gastrulation (an interesting comme on the Dalcq-Pasteels theory, cf. p. 220). In the later explants the hea structures were commonly better formed than the trunk ones, no doubt becaus being made first, they are the less dependent on the migration of the blastoder over the yolk. In later stages the main morphogenetic function of the yolk doubtless mechanical.

Oppenheimer's experiments[2] were the logical converse of those made O. Hertwig[2] in 1899, who rendered amphibian eggs meroblastic by centrifugi vn the yolk-platelets into a solid mass; animal pole cleavages occurred but trulation was impossible. This has also been done by the use of colchicine (see ;09; Keppel & Dawson). She subsequently found[7] a curious capacity for rphological without histological differentiation in presumptive tail buds ated at an early stage from fish blastoderms. After the 4–8 somite stage, vever, capacity for self-differentiation set in; this could only have been due he passage of some influence around the germ-ring from the embryonic axis.

'p to the present time little biochemical work has been done on the organisers ish eggs, but the subject has been opened by Luther's demonstration[2] that dorsal lip will carry out inductions (though weakly) after being boiled, and t the brain tissue of a young fish of the same species, as also the liver tissue ı newt, are effective inductors.

Vith regard to the formative movements of gastrulation in fish, there is general eement that they cannot possibly be accounted for by differences in mitotic :. Pasteels[3] has published data for the trout showing that there is no significant 'erence between the mitotic rate of the various parts of the embryonic shield, ugh it is higher there than in the extra-embryonic area:

	Mitoses/c.mm.
Embryonic area: average	375
Ranging from	
posterior node (dorsal lip)	317
to	
cephalic part of neural axis	475
Extra-embryonic area: average	260

ese conclusions seem to be supported by the results of American workers on *ndulus*, *Coregonus* and *Gambusia* (Richards[1]; Richards & Porter; Richards & ıumacher; Self). The blastoderm certainly thins very greatly during expansion.

Second-grade organisers in the development of fishes have not as yet received ch attention. Under eye-cup and lens induction (see the review of O. Mangold[8]), remarkable results of Werber should be mentioned. After treating *Fundulus* ; with various semi-toxic agents, such as dilute solutions of acetone, well-erentiated but completely solitary eyes with lenses appeared on the blastoderm, ıway in some cases from the main embryonic axis (see Fig. 186). Whether this ue to some kind of fragmentation of an already determined region of the toderm, or to the liberation of eye-cup inductor at an unusual place, is not wn. As regards the problem of lens induction, we have already seen that ncl's case of spontaneous lens without eye-cup played a historical part in the elopment of our knowledge of lens induction (see p. 291). Such lenses lacking -cups have often been reported (review of O. Mangold[8], cf. Fig. 187). In empting to explain their presence Werber believed that a fragment of eye-cup vays accompanied them, though it might be too small to be detected; but a ter explanation is at hand in the hypothesis that the restriction of lens inductor the eye-cup is not a *necessary* condition, and that the lens inductor may be nd free in the head mesoderm, as indeed the experiments of Okada & Mikami 'e proved is the case. Free fish lenses appear not to occur outside the head ion, but several small ones may arise side by side. Direct proof that the fish eye-cup induces a lens in presumptive epidermis has not yet been given. Corr induction may apparently take place from the lens alone, as Werber found ca in which the cornea had formed properly over free lenses. Wolffian regenerati in the fish eye remains doubtful.

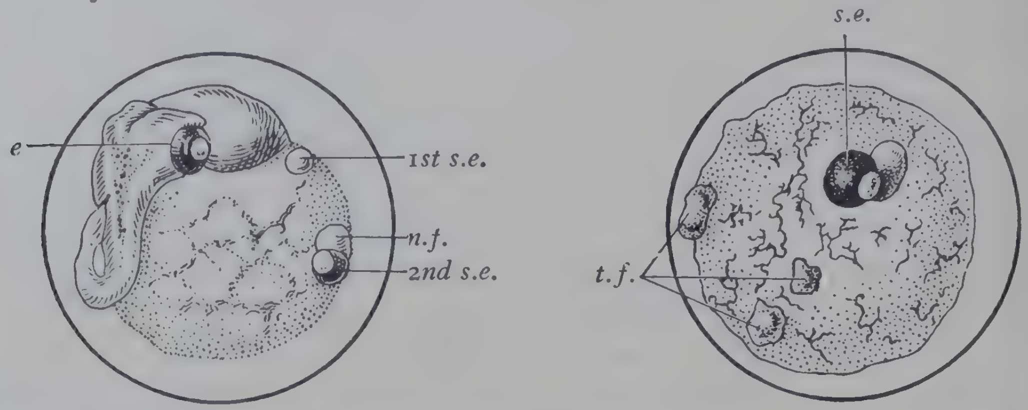


Fig. 186. Development of solitary eyes on fish blastoderms. *e.*, eye of embryonic axis; *s.e.*, solitary eye; *n.f.*, neural fragment; *t.f.*, tissue fragments.

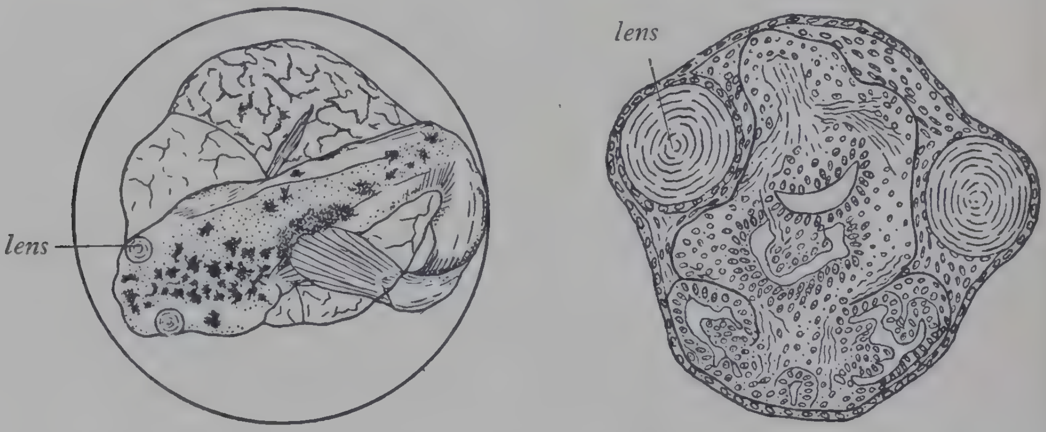


Fig. 187. Formation of lenses in teleostean fish embryos in the absence of eye-cups.

Lastly, according to Filatov[7], the induction of the auditory capsule by t ear-vesicle does not occur in teleostean fishes. The question of the induction lateral line sense organs by the lateral line nerve in fishes is complicated, a the recent paper of Bailey on this should be consulted.

2·44. Determination in Birds

The localisation of the presumptive areas on the avian blastoderm has alrea been given (p. 324). But the morphogenetic movements by which these masses material move into their proper places are still obscure and have given rise to exceptionally large amount of controversy (Gräper[2]; Wetzel; Kopsch; Marbac all cited and discussed in Pasteels[15]; and Jacobson). The blastoderm consists two parts, the transparent *area pellucida*, surrounded by the opaque yolky extr embryonic blastoderm, which will later form the yolk-sac (*area opaca*). Brief what is seen on the surface of the flat area pellucida is first the appearance o furrow, the primitive streak, followed by its extension to a maximal length.

n begins to shorten,* and its anterior end (Hensen's node) moves backwards lowed by the neural folds of the embryonic axis itself, as if it were drawing latter as a pencil would do (see Fig. 188). In due course the somites and the ad-fold appear on the embryonic axis. We now realise that the gastrulation ocess has been chronologically dissociated in birds as compared with the amphibia. Long fore the primitive streak has appeared, the oderm has formed, not, as used to be thought, invagination forwards at the posterior edge he area pellucida, but by a general downward gration of cells (Fig. 188 *ab*), which, according Jacobson[1], takes place from a rather well- ined area. Nevertheless, the primitive streak s represent an extremely elongated blasto- e. Cells converge towards it, partly directly, l partly in the famous "polonaise movement" m the outer edges of the area, and are in- ginated, forming the notochord and mesoderm ig. 188 *cd*) (Jacobson[2]). Towards the con- sion of this process (and not during it, as in the amphibia), the notochord

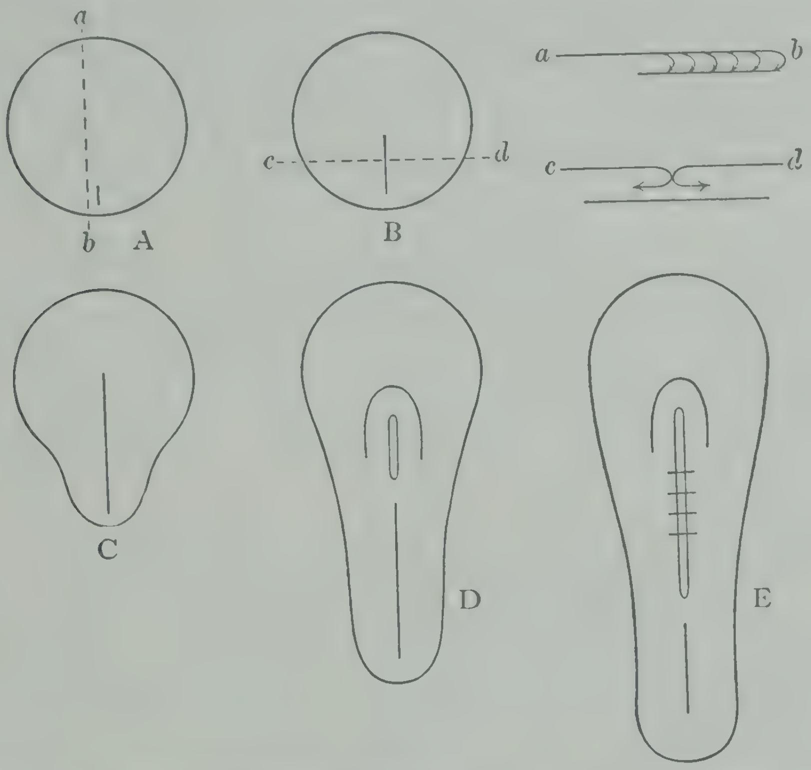


Fig. 188. The morphogenetic movements of the chick blastoderm (see text).

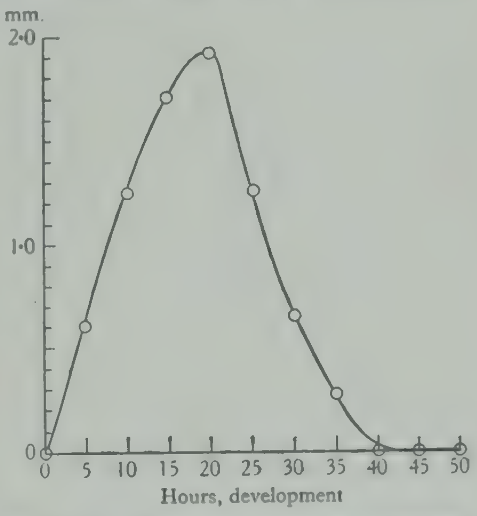


Fig. 189. Elongation and shortening of the chick primitive streak.

The regularity of the extension and retraction of the primitive streak was appreciated before its ction was understood, and the best quantitative data are still those of Edwards, collected in 2 (Fig. 189). Retraction must not be thought of as active *con*traction, what happens is rather passage of a wave of morphological change, convergent ectoderm obliterating the streak and being induced to form neural tissue.

begins to stretch, inducing first the cephalic end of the neural tube, and later more posterior parts. The withdrawal of Hensen's node is the outward and vis sign of this. It should be noted that in birds nothing corresponding to the y plug is seen, and the blastopore can never be said to be "open".

There seems to be agreement (Pasteels[5]; Derrick[2]) that the morphogen movements are not primarily due to mitosis, as the following figures show:

		Pasteels	Derrick
Mitotic rate:	primitive streak	2·43	6·1
	non-axial ectoderm	2·45	6·2

But it is not clear why the two sets of figures should be so divergent, nor d it seem to have been shown that the area pellucida thins during gastrulation much as would be expected. This subject merits a more thorough examinati especially as, according to Derrick, subsidiary centres of rapid cell-division e on both sides of the primitive streak which "feed cells into the groove and elongating area pellucida".

That inductors play a part in bird embryos parallel to their action in ot vertebrates is now well established, largely by the work of Waddington[1] and collaborators. Two principal methods have been used for studying the expe mental morphology of avian development, grafting pieces of blastoderm to anot egg at the chorio-allantoic membrane where in time they become vascularised the host's allantoic circulation; and cultivating operated blastoderms *in vitro*. T former has been much used in America, but it suffers from the criticism alrea noted (p. 175) that inductors may be present in the blood of the host.* The lat is not so free from criticism as the amphibian salt-solution explantations Holtfreter, since plasma clot has to be used as a supporting medium, bu permits of much greater operative possibilities than the former. We shall n therefore, refer further to the self-differentiations obtained by the former meth but the literature may be explored through the following references (Rawle Willier & Rawles; Hunt[1]; Murray & Selby; Stcherbatov; Dalton[2]; Hoadley

In Waddington's first explantations[1] of blastoderms of two days' incubati isolated endoderm could not be successfully cultivated, but isolated ec mesoderm yielded well-formed neural groove, notochord, somites, etc. T ecto-mesoderms, separated from endoderm, and cultivated with their mesoderm faces in contact, always both developed neural grooves, and one axis frequen induced an additional axis in the companion blastoderm. Grafts of the mid and anterior thirds of the primitive streak, placed between endoderm and ec derm in a peripheral portion of the area pellucida, induced secondary neu axes (Fig. 190). This disposed of the suggestion previously made that Hense node alone was the inducing centre. Next, an experiment similar to that wh we have described for teleost embryos was done; the endoderm was separa from the ecto-mesoderm, rotated 180° so that the longitudinal axes w diametrically opposed, and replaced for cultivation (Waddington[2]). That part

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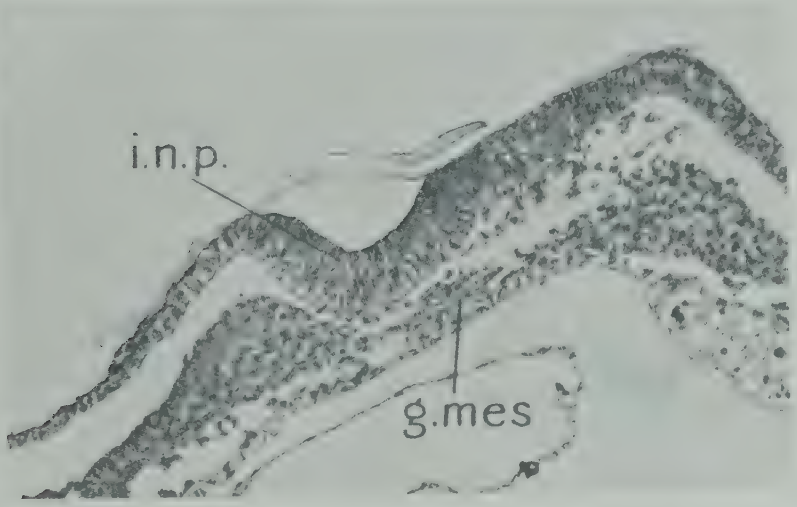
* The parallel with implantations of amphibian tissue into eye-cup or body-cavity is especi close, for Hunt[2] has obtained *bedeutungsfremd* self-differentiation of blastoderm ectoderm chorio-allantoic grafts, endodermal organs arising from mesectoderm.

ndoderm which had lain under the normal neural axis frequently induced
ndary embryo in the area pellucida in the same line as the primary, but this
ss invariably involved the formation of a primitive streak first. Endoderm
not therefore induce directly, but only by calling into being the morpho-
ic movements of primitive streak formation. In the heteroplastic trans-
ations of Waddington & Schmidt (chick/duck and *vice versa*), it was definitely
d that parts of the primitive streak which do not contain Hensen's node
e; other aspects of their work have already been considered under the head
gional differentiation (pp. 124 and 271). Homoiogenetic induction by neural
has been found (Waddington[3]). Competence for neural axis formation
s not only in the area pellucida, but in the area opaca also (Waddington[5]);
e former it falls off steadily during the decline of the primitive streak, and
e late head-fold stage it has quite disappeared, only ectodermal thickenings



190. Neural induction in the chick. *g.mes.*, graft mesoderm; *i.n.p.*, induced neural plate.

then produced by primitive streak grafts (Woodside[1]). If presumptive
rmis, presumptive notochord mesoderm, or presumptive neural plate pieces
ut between endoderm and ecto-mesoderm under the primitive streak, all
duced to form neural tissue (Abercrombie[1]), a fact which recalls the work
paschov and Töndury on the amphibia, already described (p. 170). If
itive streak pieces are placed in this position, a "struggle" between the two
taking various forms, occurs (Abercrombie & Waddington). The previous
o-posterior axis of the graft may be reversed, its presumptive regional
rentiation may be shifted so as to correspond to that of the host, or its tissues
be incorporated into those of the host; on the other hand, if the axes "side-
", the graft may perform an induction on the host, either by mesodermal
ation of new neural tissue, or by starting a new primitive streak and all that
implies. The converse of Abercrombie's experiment (making presumptive
derm into neural tissue), namely, making presumptive epidermis into
derm, can also happen (Waddington & Taylor), if a fragment of ectoderm
planted into the primitive streak itself.

Relatively little work has been done on the biochemistry of the primary orga
in the chick, but simultaneously with the first work of this kind on amphib
was found by Waddington[6] that primitive streak would still induce after ha
been killed by boiling (see Fig. 191, opp. p. 342). Later, Abercrombie[2] obta
good inductions with 1 : 2 : 5 : 6-dibenzanthracene-α-β-endo-succinate and o
hydrocarbons. He also showed that coagulated presumptive ectoderm was ac
These facts have been supplemented only by the experiments of Morita, who, u
boiled pieces of frog and mouse viscera, and of 14-day chick embryo tissues, indu
many fairly well-formed twins and even triplet embryos. Pieces of tissue k
by organic solvents, such as acetone, alcohol, or xylol, were about equally effec
but inductions were also produced by blocks of paraffin wax, agar-agar,
colophonium resin. The significance of this is not yet clear, but though no mi
photographs are yet available, it appears that the secondary embryos tende
form along torn edges of the blastoderm; they may therefore be regarded ra
as regulation phenomena than as inductions. Though we know that any par
the primitive streak may be regenerated after removal (Waddington[1]), Mor
agar inductions could not be confirmed by P. Gray.

One of the most interesting recent approaches is that of Mazia & Davis. If
blastoderm is irradiated with ultra-violet light at the primitive streak stage,
neural folds arising later will never close. Study of the wave-length effecti
revealed a typical steroid absorption spectrum (cf. Warburg's work identify
cytochrome oxidase as a pyrrol-protein and that of Sturm, Gates & Mur
identifying the chicken tumour agent as a nucleoprotein). Since, howe
neural differentiation was histologically unaffected, Mazia & Davis were proba
dealing with something other than the evocator itself (see p. 370 where gen
failures of the neural plate to close are discussed).

Amphibian histochemical work has had a parallel in the chick, for Jacobs
has studied the distribution of glycogen in the cells of the germ-layers. He fi
that there is a marked loss of glycogen from the cells of the outer layer, *bot*
the time of endoderm formation *and* at the time of mesoderm formation thro
the primitive streak. There would thus be a biochemical parallel betw
amphibian and avian gastrulation. Application of the Smith-Dietrich technic
however (said to reveal the presence of sterols, phosphatides or cerebrosid
brought to light a new phenomenon, namely a large concentration of th
substances in the primitive streak itself.* There must thus be an increase of th
in the cells approaching the streak and a decrease in the cells as they move a
laterally in the mesoderm. This decrease does not seem to take place, howe
in the head-process mesoderm, moving cranially and about to induce the ante
part of the nervous system. Use of the Ciaccio modification enabled Jacobson
state that these substances are steroidal rather than lipoidal in constitution.

It is also said that a crescentic area anterior to the primitive streak (roug
corresponding to the presumptive neural area of Jacobson[2] and Pasteels[9,15]) a
stains darkly with the Smith-Dietrich technique. If this is confirmed, it will be
first indication that biochemical differences may exist between the presump
areas on fate-maps.†

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* The concentration of these substances may affect conclusions as to cell streams drawn f
movement of dyes with lipin affinity, such as nile blue, as Jacobson warns.

† At this time also, or somewhat later, there may be differences between the regions in the na
of their ash (Horning & Scott).

we have seen (p. 203) the organisation centre of the amphibian gastrula has ·d to be a region which reduces certain dyes more rapidly than other regions ho[1]). Experiments of this kind have been made on the chick embryo by n, who finds that with janus green, reduction occurs first at the edges of the itive streak and around Hensen's node; later in the fore-brain and neural ·e. This is in agreement with the amphibian results. Preliminary tests by n also indicate that the nitroprusside test for fixed –SH is given most gly by the chick's organiser region, again in agreement with J. Brachet's ngs on amphibia[18]. Barnett & Bourne have studied the distribution of bic acid in early development histochemically. At first only in the extra-·yonic blastoderm, it appears in the nervous system on the fourth day; ıps because at earlier stages the supply cannot keep pace with the utilisation. ıack knowledge of the metabolism in the various regions of the blastoderm; nethods used up to the present (Needham[7]; Philips[2]) have been too crude ·tect any local differences.

·e come now to the second-grade organisers in chick development. The ical self-differentiation experiment of Strangeways & Fell on the eye gave ›urse no information regarding the eye-cup inductor. Hoadley's work[1], in h, after transplantation of presumptive eye district to chorio-allantois, eye- were first not formed at all, and then more and more perfectly formed, rding to the advancing age of the donor blastoderm, suffers from the general :ism of chorio-allantoic work. But it is probable that the eye-cup is induced .e fore-brain and head mesoderm as in amphibia. Lens induction occurs much amphibia* (Reverberi; Waddington & Cohen; van Deth). Lens competence ars to be very widespread (Danchakova[1]; Alexander), and as many as five :s may be induced by a single transplanted eye-cup. Van Deth finds that the of competence shrinks progressively, as in the case of the amphibian gill ;04). The inductor acts on the competent ectoderm just as well from the ide as from the inside. Wolffian regeneration can occur (Alexander). Other ·d- and third-grade inductions have been investigated by Waddington & n, who found that fore-brain may induce a nasal placode in non-presumptive erm, and by Szepsenwol[2] and Waddington[15], who studied the induction of ar-vesicles. The former identified the acoustico-facial ganglion as the ear :tor, but Waddington showed that though it may have an effect it is not the nductor since the neural tube at ear level will also act.† Ear competence is nt around the presumptive ear district, and even (according to Szepsenwol[1]) .e endoderm. The characteristic head curvature of the chick embryo is tly dependent on heart differentiation (Waddington[14]), and so, apparently, .e formation of the liver (Willier & Rawles[2]).

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* cording to Waddington & Cohen, the chick, as regards self-differentiation of a lentoid ecto-al thickening after removal of the optic cup, belongs to Group 2 (p. 292), but these thickenings ot at all like lenses, and may be due to a trace of the inductor remaining behind (see p. 294).

† ·re the rare case reported by Goldby is of interest. Not one ear-vesicle only, but a set of ermal placodes in series with it, appeared on the side of the head of a chick embryo. If were traces of the acoustico-lateral system of the anamniota they were more probably due atavistic inductor distribution in the neural axis than to an atavistic competence distribution, ıe latter is normally so widespread.

2·45. Determination in Mammals

Naturally, work on organiser phenomena in the mammalian embryo has be much delayed by technical difficulties. A remarkable lack of serological specifici as between birds and mammals, first discovered by Japanese investigato (Kiyono & Sueyoshi; Hiraiwa), has helped, however, to overcome this, f Nicholas & Rudnick[2] in 1933 succeeded in cultivating very young rat embry on the chick chorio-allantois. If a good placental circulation was achieved, goo correlative development followed, but if the tissues were vascularised directly b the allantoic circulation, excellent histological differentiation might occur, b the topographical relations would be chaotic. At the same time Waddington Waterman cultivated rabbit embryos *in vitro* on chick plasma clots, obtainin brains, hearts, neural tubes, and somites, starting with primitive streak stage In the following year Waddington[7] obtained inductions in rabbit blastoderms b chick primitive streaks, and later *vice versa* (Waddington[11,16]). But inductions b rabbit primitive streak in rabbit have not yet been obtained, though Törö[3] h produced striking homoiogenetic inductions by neural tube in rat embry (Fig. 192, opp. p. 342).

Future progress in this important field will no doubt be furthered by the ne apparatus of Nicholas[2] for maintaining the development of the mammalia blastocyst *in vitro* by a circulating medium. Waterman[2]; Nicholas[2]; Nicholas Rudnick[1,3] and others have cultivated mammalian blastocysts in mammalia plasma, but so far without advancing our knowledge of the inductions proceedi in them.

Work analogous to that of Richards and his collaborators (see p. 329) has bee carried out on the rat embryo by Preto, but no correlation between mitotic ra and morphogenetic movement was found.

The mammalian egg (rodents) has been investigated in the spirit of Spek work with vital stains by Arnold (cf. p. 133). In conformity with its regulative character no differences between the regions of the egg or the morula cou be detected, but as soon as the blastocyst was formed, the cells of the embryon mass gave reactions quite different from those of the blastocyst wall. In so f as could be ascertained from the rather uncertain pH indicators which alon would penetrate into the cells, the embryonic mass was on the alkaline side neutrality and the blastocyst wall cells on the acid side (see p. 141). The fluid the blastocyst cavity appeared to be above pH 8·5.

2·5. Temperature and morphogenetic processes

Acceleration of embryonic development with rise of temperature is in itse a banal phenomenon, and its mathematical analysis leaves us only with furth enigmas. It will not here be reconsidered (cf. *CE*, pp. 515 ff.).* In so far the whole pattern of the organism reacts alike, accomplishing development mo quickly, little further understanding of the fundamental processes is attained. On

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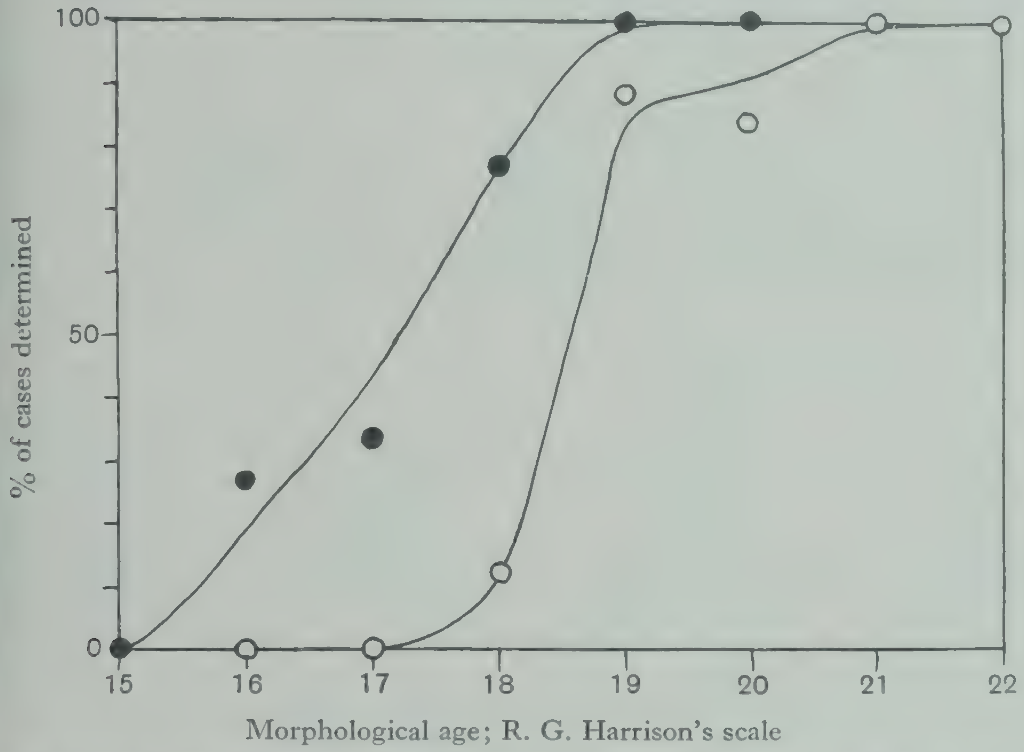
* Among the most careful recent papers here are those of Atlas[1] on amphibia; Worley; Price J. A. Moore[1]; Merriman on fishes; Pritzker on the chick.

temperature changes dislocate the developmental processes in an observable
can any progress be made. At the same time it may be useful to show
specially sensitive periods exist with regard to temperature, one process, for
ple, being much more sensitive to temperature change than another.

r these reasons, the most interesting work on temperature effects in morpho-
sis (the determination problem) is probably that of Twitty[1]. The determina-
process may be shifted experimentally in time, and so thrown out of gear
the growth process. Twitty found that the polarity of the ciliary beat of the
lermal cells of the amphibian embryo is normally determined during the
re of the neural folds (cf. Austoni), for the cilia of ectodermal grafts rotated



93. Influence of temperature on time of determination of ciliary polarity in *Amblystoma*. Embryos kept: at 15° C. ○, at 7° C.; ● before the operation.

gh 180° before that stage beat in the same direction as the cilia of the adjacent
ectoderm (neighbourwise), while ectodermal grafts rotated and transplanted
retained their original direction of ciliary waving (selfwise). But in embryos
ed to develop at lower temperatures than normally, Twitty found that the
y polarity appeared to be determined at a much earlier stage relatively to
ral morphological development. As Fig. 193 shows, the percentage of trans-
s showing determined ciliary polarity rose to its maximum earlier relatively
orphological age in the cooled embryos than in those maintained at normal
perature. If, then, the determination process itself could be thought of as
ng a temperature characteristic, it would be lower than development, i.e.
le morphological differentiation, as a whole. Conversely, a reaction inhibitory
e determination process would have a temperature characteristic higher than
of development as a whole. The fact that determination can be affected in

this way by temperature is a hopeful prognosis for a better physico-chem understanding of it.

Another interesting fact is that according to Lopaschov[8], neural inductio dead inductors is much more effective at low temperature than at high. explanation of this is unknown. But on the contrary, regulation in hom plastically grafted eyes in axolotls is less effective at low temperatures tha high, or may even fail altogether (Twitty[7]).

Differences of temperature characteristic between different stages of devel ment have long been reported, e.g. for the development of the sea-urchin emb (*CE*, p. 507), though they can hardly perhaps as yet be said to be established. A fa convincing example of this type of work is that of Ljubitzky & Svetlov, who no

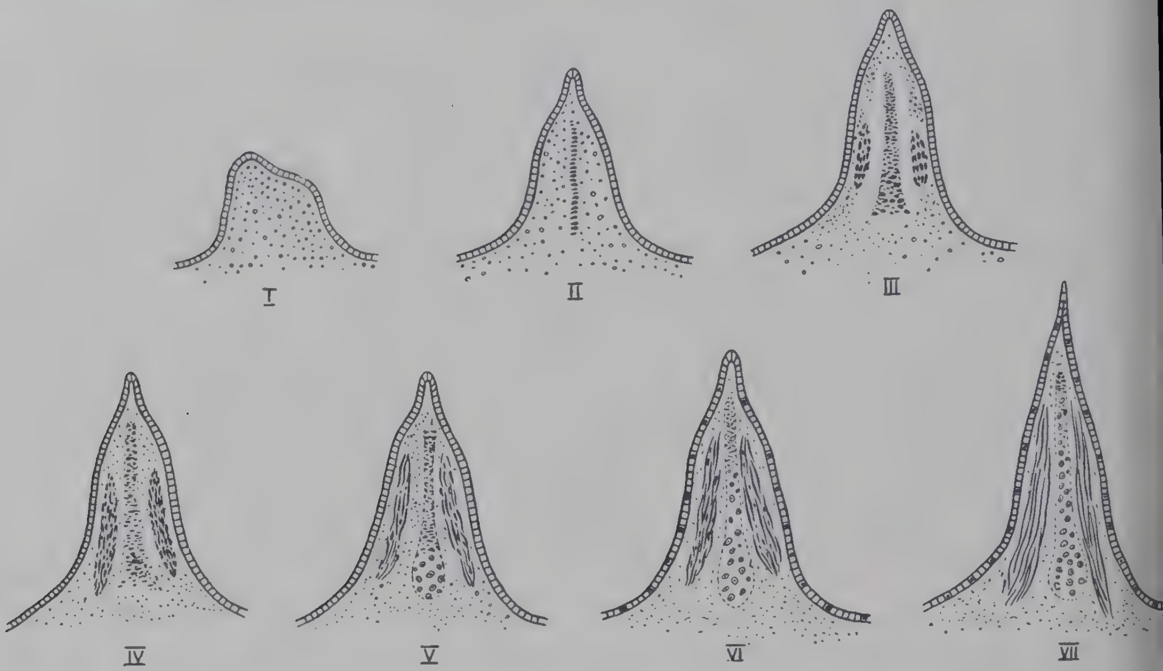


Fig. 194. Relative responses of the pectoral fin of the trout to temperature at different phase its development.

Stage	I	II	III	IV	V	VI	VII
Q_{10}	2·3	1·0	5·9	3·0	1·0	1·0	1·0

the relative responses of the pectoral fin of the trout to rise of temperature different periods of its differentiation. Their results are summarised in Fig. 1 In the earliest stage, that of the undifferentiated blastema, the temperatu acceleration was considerable, then it dropped, to rise again at the period of ra histological differentiation of the skeleton and muscle masses. When the his logical differentiation was over, the effect of temperature again became negligib Similar, but less clear-cut results, have been described by Ljubitzky for t period of blastoderm formation, yolk encirclement, and axiation in the trout, a by Latinik-Vetulani[1] for the early development of the amphibian embryo. H results are difficult to interpret owing to the high supra-maximal temperatu used; Hoadley[7], studying the same kind of material histologically, noted th persistence of the blastopore and microcephaly, due to some interference w the head-organiser, were the most common results.

good deal of work has been done on the *localised* application of heat to the hibian embryo, in which the embryo is subjected to a "temperature gradient".* main effect of this seems to be an acceleration of cleavage, so that the usual rences between the size of animal and vegetal pole cells in the blastula, for nce, are lessened or abolished. Later stages may show fairly marked asymry, one neural fold being larger than the other. Eventually great differences veen the development attained by head and tail ends may be found (Vogt[3]; ley†; Dean, Shaw & Tazelaar).† But the only investigator who has drawn conclusions about determination from such experiments is Gilchrist. His experiments (Gilchrist[1]), did not go beyond the asymmetries, puckerings, etc. he previous work. In later work, however (Gilchrist[3]), he adopted the plan eating first on one side, then on the other, so that each side should have been lly subject to the acceleration produced by rise of temperature, though this ld have occurred at different times. The gradient was usually applied for t 50 hr., with a reversal at the mid-point of the period, and a succession of e periods on different batches of eggs covered the whole of development n the earliest blastula stage to just before the closure of the neural folds. The ment now ran as follows: if one-sided heating produces an increase in the of the neural fold on that side, then the period during which the heating applied must have been that at which the determination of the neural plate going on. And so similarly for other structures. The results may be tabulated s:

Process	Period during which determination would be proceeding
Morphogenetic movements of gastrulation	Cleavage stages—early blastula
Determination of neural plate	Early blastula—late blastula
Determination of size of brain and head	Late blastula—half-moon gastrula
Determination of pronephros	Full-moon gastrula—open neural plate

re is thus a certain contradiction between these results and those already ribed (p. 154), for while, according to the accepted view, the determination of the neural plate proceeds during invagination by the action of the archen-n roof, here it appeared to take place before invagination had begun. Gilchrist[3] osed, as an explanation of this difference, that we should distinguish between k" and "strong" tests of determination. "Strong" tests are those in which neighbourwise or selfwise behaviour, and hence the determination of an yonic part, is tested by transplantation to an unusual region of a host. ak" tests are those which do not subject the part to such new surroundings; he method of reversed thermal gradients. If then, the determination process an appreciable time from its first onset until its final completion, we should ct that the stronger the test, "that is, the more potent it is in bringing the loping material into new and active relationships, the later will the time of mination appear to be". But this distinction between "strong" and "weak" is not acceptable. The definition of determination is the firm capacity of issue for self-differentiation, from which it will not be deterred *whatever its onment*, if it lives. "Weak" tests simply do not test this at all.

is unfortunate that Gilchrist published no clear histological sections of his nmetric neural plates, for we do not really know what a "larger" neural fold

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e have already noted (p. 188) that extreme applications of this have been made in the effort oduce inductions, but so far without success.

ymmetries of various kinds, interpreted in gradient terms, have also been obtained by the f a variety of unrelated chemical substances in the water surrounding amphibian gastrulae sky, Tazelaar & Huxley; Svetlov[2]).

on one side means. It may be that more cells are derived from the adjoin presumptive epidermis, but it might also merely mean that there is less floo these plates, i.e. a simple distortion of the normal cross-section.

For the sake of argument, however, let us grant that Gilchrist's larger ne folds contain cells which should have been epidermis. It is true that for c at the border of the neural area, determination of the size of the plate is the sa thing as determination of the existence of the plate. But there is nothing to pr that the effect of localised warming on determination is immediate. It mi affect processes preliminary to evocator liberation. Or it might exert a dela effect on such border-line cells so that the inducing stimulus, when it ca later on, spread further than usual in that direction. In other words, competen not determination, would be affected.

Gilchrist's experiments, therefore, have not proved that determination i process requiring an appreciable amount of time, and that there is an intermedi stage of labile determination. But to quarrel with this view would be to disp a truism. All statistical curves of determination processes show an intermedi period when sometimes one result is obtained, sometimes the other. This h been pointed out by Brandt some five years before Gilchrist's work, as the res of a consideration of the determination of limb-buds (discussed on p. 666), whe the various spatial axes are determined at different times. Exactly how quick the determination process proceeds in a given tissue is, of course, a matter f much future research. A new notation is really required, describing for any giv piece of tissue at any given developmental stage its subsequent behaviour in possible new situations, including self-differentiation *in vitro*. This would invol a list of its competences.

Second-grade organisers have been the subject of a few experiments in whi temperature was studied. Lehmann[9] found that the action of trichlorbutyl alcoh in suppressing lens formation in amphibia (see p. 224) was maximal at 14° C.; le marked at lower and higher temperatures. After some earlier work, Nakamur has established that Wolffian regeneration is especially sensitive to temperatur in the oriental species studied by him (*Triturus pyrrhogaster*) winter temperatur of 16° C. inhibit it completely. Light intensity and nutritive conditions were rule out. Further relevant facts will be referred to later (pp. 450, 516).

2·6. Genes and organiser phenomena

We now enter upon the widely ramifying subject of the relations betwee genetics and embryology. It may be separated into a number of divisions. I the first place, it is known that organisers will act across genetic boundaries, i.e material of quite different genetic constitution will react to the same organise stimulus, though the ways in which it does so will differ according to this constitution. Organisers must indeed be regarded to a large extent as the intermediary mechanisms between the gene equipment and the final form an properties of the developed animal. It is therefore important to know how gene and organisers are related in normal development. But in order to do thi advantage must be taken of those instructive cases in which abnormal development occurs. If development is only very slightly abnormal and leads to a change organism, we may trace the change back to a mutation in the genome but th case may be difficult to analyse embryologically owing to the smallness of th ge. All such changes are not small, however; hence the importance of lethal ıb-lethal genes. At the same time advantage must also be taken of the rmalities which occur when the nuclear substance of either gamete is rimentally interfered with before or about the time of fertilisation. There lso to be considered various interferences with the normal plan of growth of the parts of the body, which we may classify as disturbances of the iduation field.

the following discussions we shall no longer be in a realm where the end-t is purely morphological. It is difficult to consider the actions of the genes h cause morphological effects in abstraction from the actions of those which rn reaction rates, the expression of which is in terms of the production of ıred substances, or the accumulation of chemically defined storage materials. ıe first section, then, will deal with the action of organisers across genetic daries, that is to say, the effects of an inductor of one genetic species upon etent tissue belonging to another. From pp. 171 and 301 it will by now be nt that the primary organiser of vertebrates, and at least some of the ıd-grade organisers, have no species-specificity. A piece of chick primitive k in the blastocoele cavity of an amphibian embryo inducing a secondary yonic axis can obviously induce only an amphibian one. In such extreme it is easy to see that the induction can only be "autonomous", not "com-entary". But in cases where the inductor and the competent tissue are closely related, it might happen that the inductor would overcome the tic determination of the reactor in favour of its own characteristics.

51. The action of organisers across genetic boundaries

ıe question was first posed by Spemann[10] in 1921; he found that when ımptive neural tissue of *Triton taeniatus* was grafted into the presumptive listrict of *Triton cristatus*, it helped to form the gills while at the same time ıing its own histological character. Later Rotmann[3], a member of Spemann's l, devoted himself entirely to following up this interesting line of work. xperiments on the gill were first confirmed (Rotmann[1]; Schmidt[10]) and ded to the limb buds,* but a more decisive case was found in balancer tion (see p. 302). If an interchange was made between presumptive balancer rm of *T. taeniatus* and *cristatus* the balancers subsequently formed, though normally induced (by the host's endoderm), "showed exclusively the cters of the species from which the ectoderm had been taken". Balancers *status* not only have a thickened ectoderm compared to the others, but they se more endoderm and point forward, while the others point backward nann[2]). Lens-forming ectoderm interchanged between the two species behaves

oderm of *cristatus* put into the limb-bud district of *taeniatus* formed a limb composed of *us* ectoderm and *taeniatus* mesoderm; it had the shape of a *taeniatus* limb. On the other *cristatus* presumptive mesoderm was so implanted that it invaginated with the *taeniatus* erm; thus a limb composed of *taeniatus* ectoderm and *cristatus* mesoderm was produced *aeniatus* embryo); it had the shape of a *cristatus* limb. Hence the mesodermal part of the s much more important in determining its form than the ectodermal part (Rotmann[1]; on[10]). Such ectodermal grafts persist in showing the characteristics of the donor's skin long ıetamorphosis (Rotmann & McDougald).

similarly; on induction it will give a lens, but the size of the lens will b accordance with the species from which the competent ectoderm is derived, will not be related to the size of the inducing eye-cup (Rotmann[4]).

It is thus clear that the competent tissue gives the required answer corre but "in its own language", to use an anthropomorphic analogy. The striking case of the kind so far described is that of the mouth parts of amphi Urodele larvae have genuine teeth in their mouths, of the same structure origin as the teeth of all vertebrates; those of anurans, on the other hand, h only horny jaws and stumps quite unlike teeth. If urodele and anuran ectod could be so exchanged as to come under the influence of the head endoderm mouth induction, what would be formed? Correspondingly, urodele lar possess, somewhat laterally and behind the mouth, a pair of balancers, w anuran larvae have two areas of thickened ectoderm secreting a sticky substa and called suckers, behind the mouth in the median line. Again an exchang urodele and anuran ectoderm was required.

The second question was answered before the first one. In 1932 Speman Schotté transplanted presumptive epidermis from any region of an early gastr of the frog *Rana esculenta* into the balancer district of *T. taeniatus*, with the res that very well-shaped and functional suckers were formed* (Fig. 195). The conve experiment was made by Rotmann[3], who moved presumptive ectoderm fro young *Triton* gastrula to the sucker district of *Bombinator pachypus*, with result that well-formed balancers were produced (Fig. 196). In one case, wh the graft was one-sided, a sucker was formed in its normal place in the host fr host material, and on the other side a balancer was formed from graft materi Many other cases of this kind have since been obtained (e.g. by Schmidt[8]). therefore have in the endoderm of these districts a common stimulus produci a different result according to the competence of the tissue subjected to Spemann has written "one might assume that the stimulus releasing the develo ment of either organ would still be the same as in that organ of the ancest from which the two recent organs have been derived; whereas the reaction the ectoderm would have changed" ([17], p. 359).

In this connection a certain experiment of Mangold's[10] is very instructive. T axolotl larva, though a urodele, does not possess a balancer. Hence ectode from a suitable area of the axolotl larva would not produce a balancer wh grafted into the balancer district of *Triton*. But on the contrary, presumpti balancer ectoderm from *Triton*, removed long before determination could ha occurred, formed a balancer after being grafted on to the appropriate region *Amblystoma*. Therefore it seems certain that in the course of evolution t ectodermal competence, but not the endodermal inductor, has been lost in t axolotl.

The first question, that of the teeth as opposed to horny jaws, was settl by Schotté (cited in Spemann[17]) and by Holtfreter[13,14]. Genuine teeth will produced in urodele ectoderm after the action of anuran mouth-organiser, t normal effect of which is horny jaws (Fig. 197). Horny jaws will be produced

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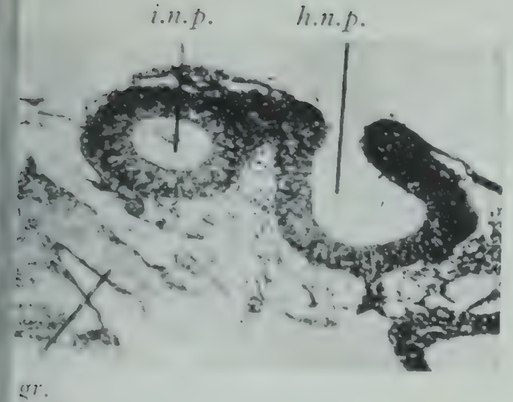
* Often confirmed, as by Schotté & Edds.



1. Induction by dead chick organiser in the k. *h.n.p.*, host neural plate; *i.n.p.*, induced al plate; *co.gr.*, coagulated graft.

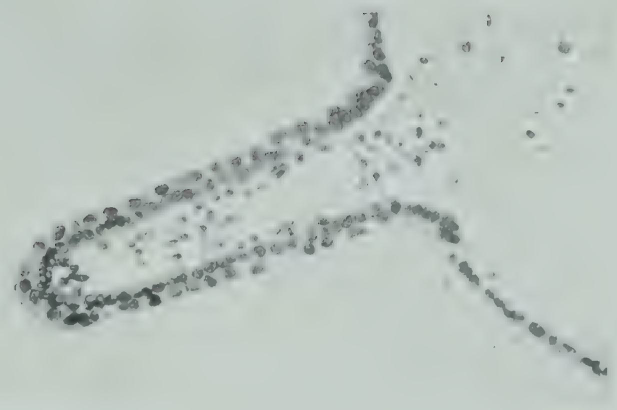
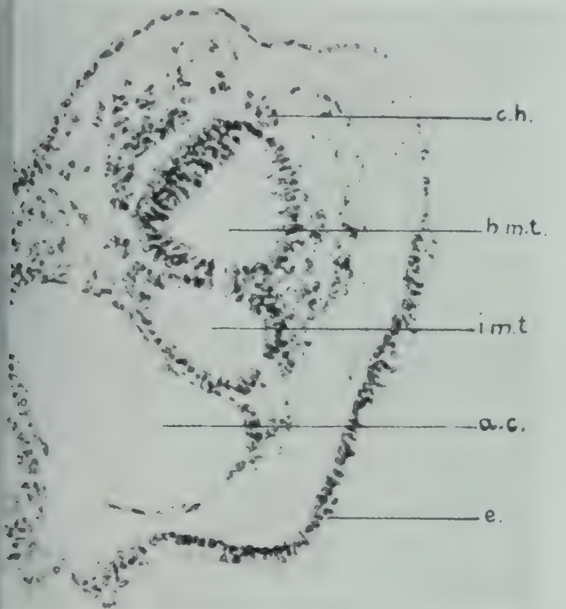


Fig. 196. Balancer formed in urodele ectoderm on the anuran body, by the action of the anuran sucker inductor.



192. Homoiogenetic induction in mammals t). Implantation of a neural plate fragment 9-day embryo into the proamniotic cavity 7-day embryo, followed by 70 hr. cultiva- *in vitro*. *c.h.*, notochord; *h.m.t.*, host ral tube; *i.m.t.*, induced neural tube; *a.c.*, iotic cavity; *e.*, endoderm.

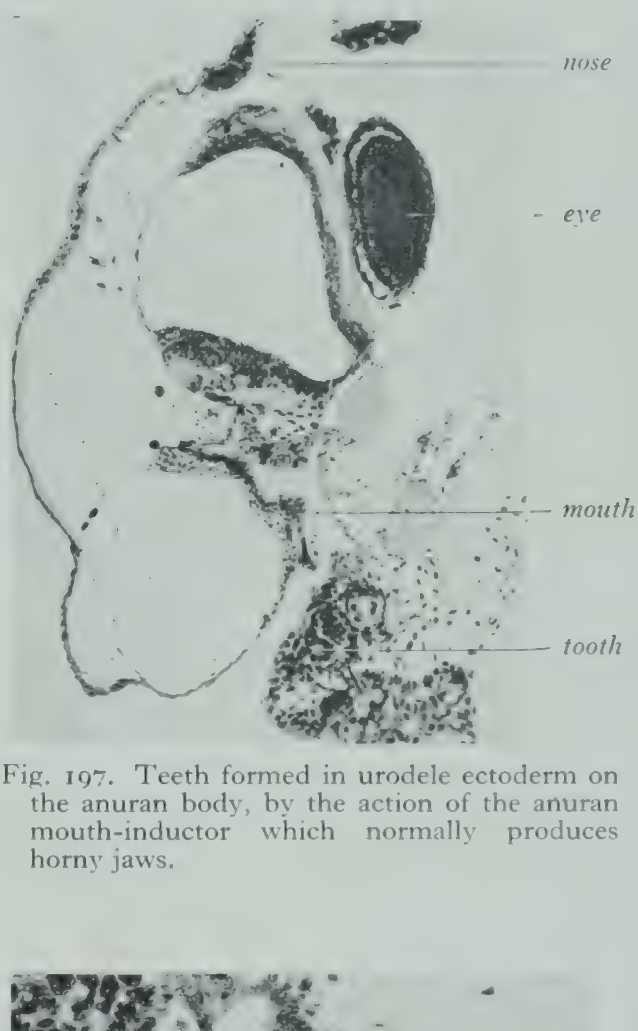
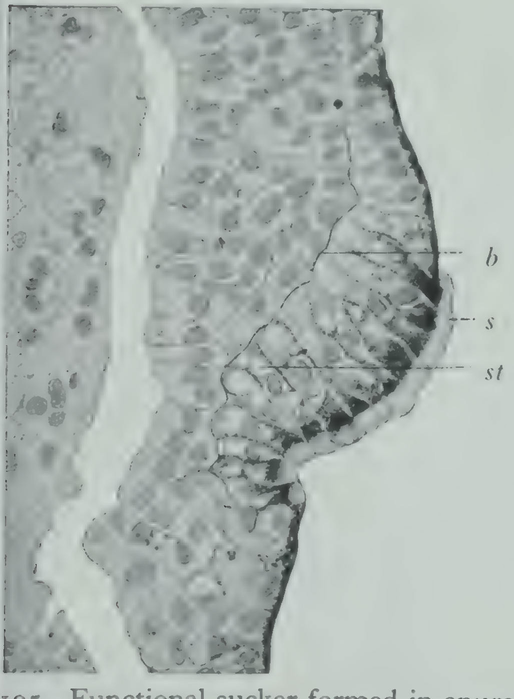


Fig. 197. Teeth formed in urodele ectoderm on the anuran body, by the action of the anuran mouth-inductor which normally produces horny jaws.



195. Functional sucker formed in anuran toderm on the urodele body, by the action of e urodele balancer inductor. *b*, boundary the outer ectodermal layer; *s*, secretion; , droplets of secretion.

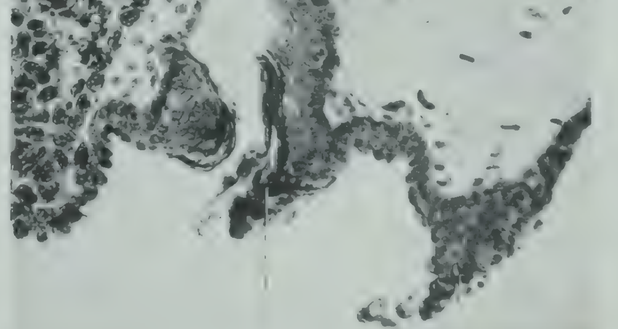


Fig. 198. Horny jaws formed in anuran ectoderm on the urodele body by the action of the urodele mouth-inductor which normally produces teeth. *hpj*, horny prominence of jaw; *hpl*, horny prominence of lip.

n ectoderm after the action of urodele mouth-organiser, the normal effect ich is teeth (Fig. 198). It is thus certain that competences for organs which ost does not possess at all may react to stimuli which normally act upon competences. “It is as if”, says Spemann[17], “the cue were quite general— th armament’, and at this cue the needed organs would be formed, as they rovided in the genotype of the reacting tissue.” In terms of modern ledge of biochemical specificity, this may well mean that the same key chemical substance, the inductor) may be able to unlock several different .* This may also have a bearing upon the induction of the limb-bud by esicle or nasal placode, for there is no reason for supposing that the normal tors in those organs may not be able to unlock the limb-bud system, ugh naturally such an overlap of specificity never comes into play in the ally developing organism, and does no harm there.

52. Genes and the later stages of individuation

e experiments just described have given a clear answer to the question ner the determination of a tissue of one genetic composition can be brought by the action of a tissue of another genetic composition. Yet with the first ogical differentiation of an organ the development of that organ is not ed. Growth processes have to occur, and the onset of physiological function be successfully passed through. The further question may therefore be in the case of a transplanted organ; would its growth rate and its physiol characters be determined by its host or by the donor? In other words, have seen, a piece of tissue of one genetic composition will behave *mäss* (neighbourwise) to tissue of another genetic composition as regards rgan induced, but *herkunftsgemäss* (selfwise) as regards the “style” of ew organ, which will be in accord with the graft’s genetic composition. If an organ fully determined as regards its histological differentiation, such as or a limb-bud, is transplanted, will it continue to show the growth rate teristic of its donor, thus behaving selfwise in accordance with its genetic sition, even though this should lead to serious disequilibria in the plan host’s body; or will it be in some way regulated to correspond with the

Correspondingly, when the histological differentiation of an organ is nined, is its later physiological behaviour also determined, or can this still be d by transplantation to a host the analogous organ of which shows a different logical behaviour? It will be seen that we have here the link between the nination problem and the problem of heterauxetic growth (Section 3·2).

se questions have been the study of Harrison and his colleagues over a eriod of years. They presented certain special difficulties, for example, the lty of accurate measurements of organ sizes without killing the animal, ne complications produced by different levels of food-intake, etc. For these s the reviews of Harrison[11] and Twitty[3] must be consulted; here the require-

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ylcholine is a parallel to this. Though present in many invertebrate groups, the reaction the part of the effector systems is by no means the same. And we know of substances, 1 : 3-dimethylbutyl-ethyl-barbiturate (Swanson & Chen) or harmine (Chen & Chen) ause convulsions in mammals but depression and anaesthesia in amphibia.

ments of space limit us to a short account of the main results. Reference already been made to the classical experiment of Harrison[1] in 1898 on the heter plastic grafting of differently pigmented anuran larvae for the study of

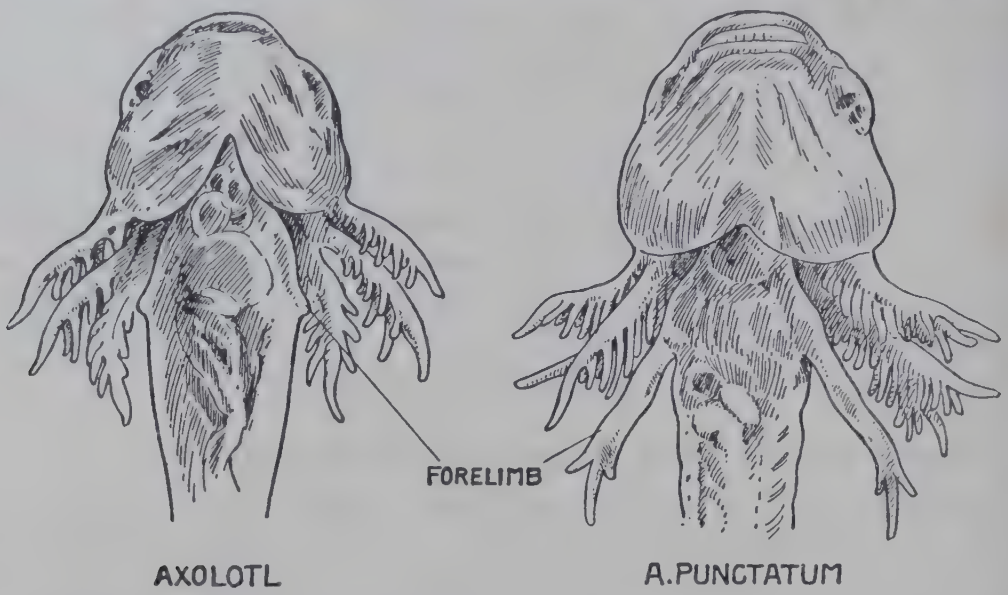

Fig. 199. Differences in limb-development at the beginning of the larval stage in the two speci of *Amblystoma, tigrinum* and *punctatum*.

development of the lateral line (p. 148); from this the later work was a natur development.

The two species of axolotl, *Amblystoma punctatum* and *tigrinum*, differ in t time of appearance of their forelimbs. At the beginning of the larval period, when the yolk has been used and the animals are ready to feed, the limbs of *punctatum* are almost fully developed, having arm, forearm and hand with two long and two rudimentary digits. The limbs of *tigrinum*, on the other hand, are at the same age only minute buds (see Fig. 199). When transplantations of the presumptive limb areas were made (Harrison[6]; Twitty & Schwind) it was found that the growth rates were quite determined, *punctatum* limb on *tigrinum* host developing rapidly and *tigrinum* limb on *punctatum* host developing slowly. Owing to the fact that *A. tigrinum* attains in adult life a larger size than *A. punctatum* there is later a secondar reversal of limb sizes.*

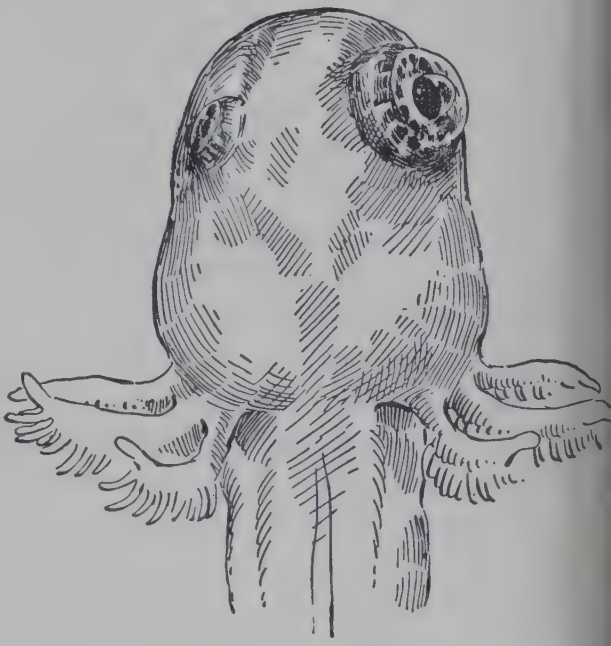

Fig. 200. Larva of *A. punctatum* carrying an eye the larger *tigrinum* species.

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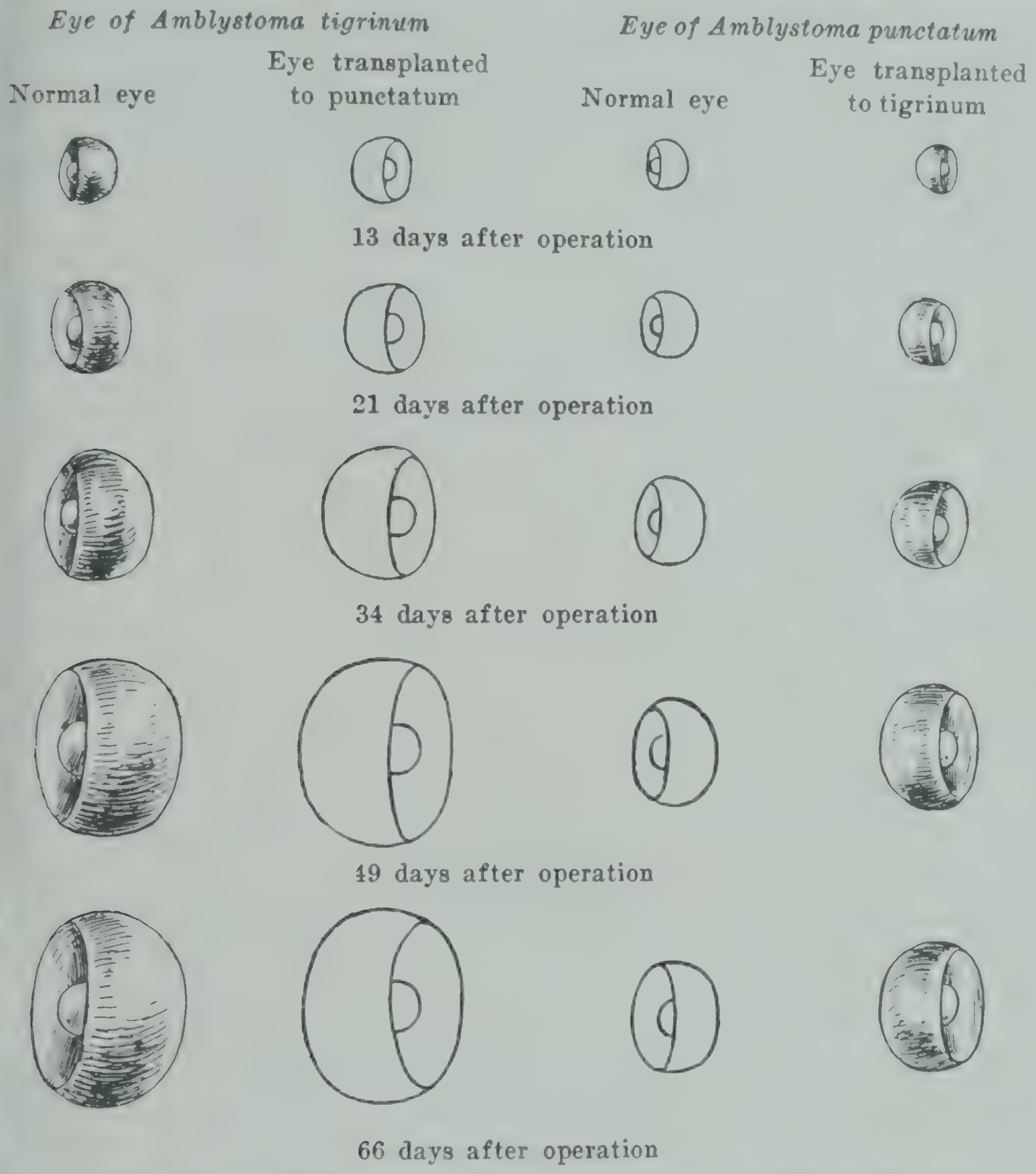
* Note that the Q_{O_2} of *tigrinum* embryo tissue is higher than that of *punctatum* (Wills).

ssentially similar results have been obtained for the eye by Harrison[9]; Twitty
chwind and Stone. The eye-cups of *tigrinum* are normally very much larger
n those of *punctatum*; Fig. 200 shows a larva of the latter species carrying an
of the former on the right side. But on transplantation the eye preserves
ost exactly the same dimensions as it would have done in the donor (see Fig.
, taken from Twitty & Schwind). Towards metamorphosis there is, however,


Eye of Amblystoma tigrinum
Eye of Amblystoma punctatum
Normal eye
Eye transplanted to punctatum
Normal eye
Eye transplanted to tigrinum
13 days after operation
21 days after operation
34 days after operation
49 days after operation
66 days after operation

201. Lack of regulation in axolotl eyes transplanted between species of different eye-size.

ery slight regulation of eye size in *punctatum* eyes transplanted to *tigrinum*.
e experiments were further elaborated by Harrison[9] who transplanted eye-cup
lens ectoderm separately and found that *tigrinum* eye-cup on *punctatum* host,
h a lens which it has itself induced from *punctatum* ectoderm, still grew more
idly than the *punctatum* eye though less so than the transplanted *tigrinum*
-cup with *tigrinum* lens. The small *punctatum* lens could therefore exert a
ain inhibiting influence. More remarkable, in the converse experiment, the

punctatum eye-cup was accelerated in its growth by the *tigrinum* lens which
had induced but which was really too large for it. Lens ectoderm alone grafte
from *tigrinum* to *punctatum* suffers induction, and produces a lens which is to
large for the eye-cup, hence the growth of the lens is retarded but that of th
eye-cup is accelerated. Conversely, lens ectoderm alone grafted from *punctatu*
to *tigrinum* suffers induction, and produces a lens too small for the eye-cup, henc
the growth of the lens is accelerated and that of the eye-cup retarded.

The general upshot of these experiments, continued by Lazarev, was therefor
that while within an organ such as the eye, mutual adjustments between the part
can occur, there is little or no regulation of the growth rate of the organ as a whol
to suit the organism as a whole. The "out-of-scale" eyes have later secondar
effects on the nervous system and muscles (Twitty[2]).

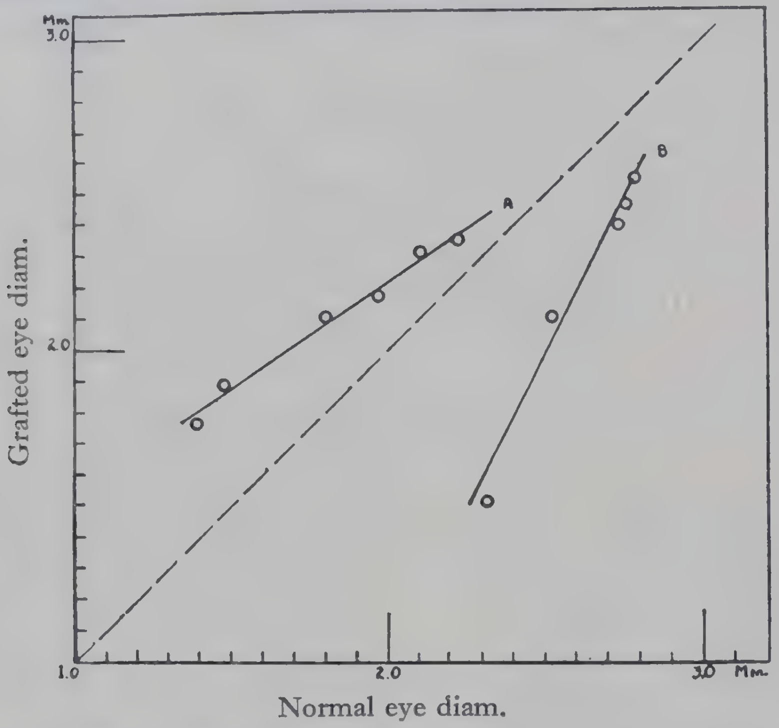


Fig. 202. Regulation in axolotl eyes transplanted between individuals of the same species but o
different age (homoioplastic transplantation). *A*, graft larger than host eye; *B*, graft smalle
than host eye.

The only conditions in which considerable regulation does seem to occu
is in reciprocal transplantations between old and young larvae of the sam
species. Twitty & Elliott removed both eyes from a medium-sized larva o
Amblystoma tigrinum and grafted one to a larger animal than the donor, the othe
to a smaller. There followed in every case an accelerated growth of the graft i
the former case ("catching-up") and a retarded growth in the latter ("markin
time"), so that after some while the normal proportions were restored. This i
shown in Fig. 202. Here, however, genetic size differences are not involved
In his review Twitty[3] explains this homoioplastic regulation effect on the basis o
the Robb partition-coefficient theory of heterauxetic growth* (see p. 536). Twitt
& Elliott showed that similar age regulations could also be observed in hetero-
plastic eye grafts though in such cases the position of equilibrium finally reache

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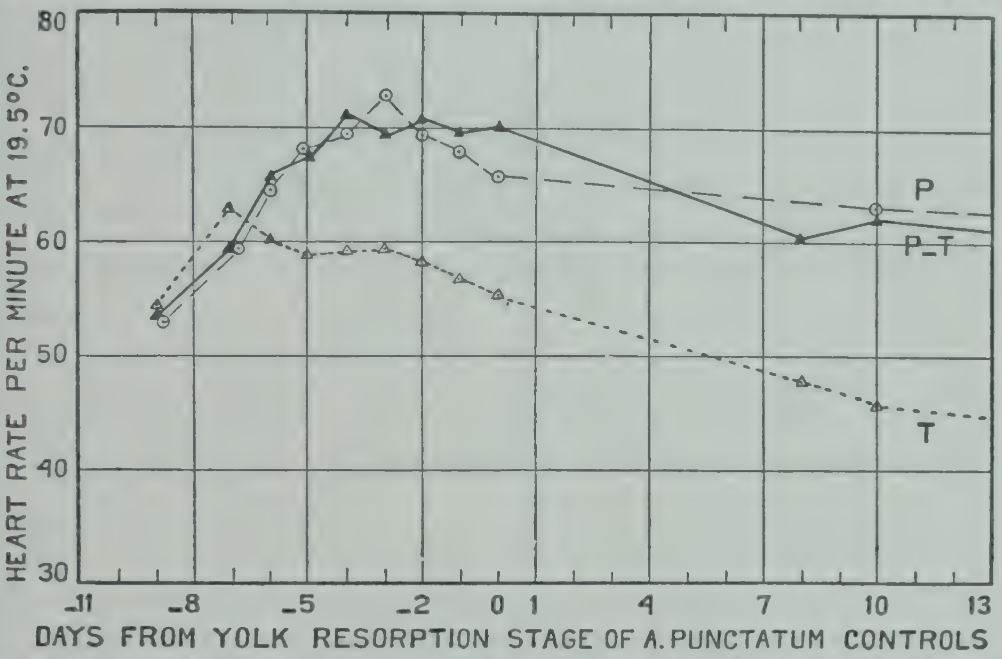
* See the explanatory blood analyses of Twitty & van Wagtendonk.

not, of course, the symmetrical one proper to the unoperated host. We ll later have occasion to note some other homoioplastic transplantations* ween younger and older animals with reference to regeneration (p. 439) and tamorphosis (pp. 449, 454, 466) phenomena, and some other manifestations the "catching-up" effect (p. 589).

The extremest cases of regulatory or neighbourwise development seem to be se studied by Detwiler[2], where the larger part of the spinal cord is transplanted. er implantation of a piece of *tigrinum* spinal cord into a *punctatum* embryo at corresponding position, the grafted cord is for a considerable time too long, thick, and too full of cells. But eventually a far-reaching regulation takes ce so that it fits precisely into the nervous system of the host. This is explained



. 203. Physiological selfwise behaviour of the pulse rate in exchanged axolotl hearts (*punctatum* grafted to *tigrinum*).

'functional differentiation" due to the modifying influence of the stimuli hing the central nervous system from all parts of the *punctatum* body. With case we link the determinative period of development with the period of ctional differentiation, and reach the utmost confines of the former.

The experiments of Copenhaver on the heart-beat rate of transplants between *blystoma* species are of particular interest, as they bring physiological function within the framework of the concepts of neighbourwise and selfwise development. At the end of yolk absorption the normal rate of beat for the *punctatum* rt is 60/min. at 19·5° C.; that for the *tigrinum* heart is about 40/min. As . 203 shows, the pulse curve of the *punctatum* heart grafted to a *tigrinum* bryo closely follows the rate of the donor species. When portions of hearts were nsplanted, it was found that the posterior part sets the pace. As Fig. 204 shows, pulse curve of the heart in a *punctatum* embryo which contained sinus and um from *tigrinum*, followed exactly the pulse curve of the *tigrinum* embryo.

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* he gill and the ear-vesicle have also been studied from this angle, but these cases are complex, the reader must be referred to Harrison's review[11].

No doubt the most marvellous case of physiological selfwise development is tha reported by Giersberg, who exchanged at an early stage the brains of differen species of toads and frogs (e.g. *Pelobates fuscus* with *Rana arvalis*). An anima when adult, possessing a *Pelobates* brain in a *Rana* body, showed the stron digging instinct characteristic of *Pelobates*.

Much of what has been described in this section holds good also of insects Bodenstein[1,13] has shown that regulation of growth-rates only occurs in transplantations between young and old animals.

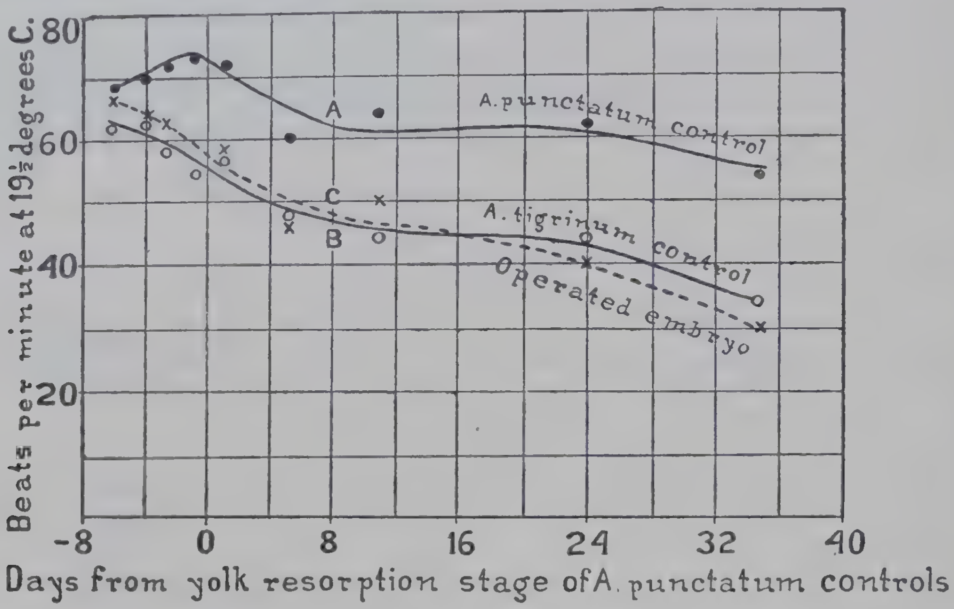

Fig. 204. Physiological neighbourwise behaviour of the pulse rate in exchanged axolotl heart (*punctatum* under the influence of sinus and atrium of *tigrinum*).

But how it is that the very bold xenoplastic and heteroplastic transplantation so far described have been possible? Is there no such thing as "species incompatibility" and immunological resistance to transplants? This we must examin in the following section.

2·63. The origin of species-specificity

This section is to some extent a digression. It is only intended to call attentio to the generalisation that tolerance to transplants decreases both phylogeneticall as one ascends the taxonomic scale, and ontogenetically as the earliest stages o development are passed through. A wide literature could be drawn upon t illustrate these statements; here we shall simply adduce a number of experiment which make the relationship clear. The phenomenon is an example of the "la of recapitulation" in its simplest form (as described in *CE*, p. 1638), namely tha the simple precedes the complex both in ontogeny and in phylogeny. The capacit of cells to live side by side though of different genetic, specific or even generi constitution, depends on the degree to which mutual protein intoxication occurs and the older a given animal, or the higher a given animal in the taxonomic scale the more certain is such intoxication to happen. For both age and phylogeneti status involve increased complexity, and hence increased specificity, of protei molecules as of morphological organisation.

aking first the phylogenetic series, a large body of work (ably reviewed by .oeb[3]) has shown that there is a graded series of reactions to foreign tissues, 1 complete tolerance through leucocytic infiltration and gradual resorption to l disintegration and sloughing of the graft. In mammals there is intolerance reen individual organisms of the same species and variety, even, although lesser degree, between individual organisms of close blood relationship. In hibia these individual differences are not seen. But in spite of the far-reaching ons which, as we have already found (pp. 156 ff.), are possible between urodele anuran amphibian embryos, it was early found by Weigl that xenoplastic lele anuran skin transplantations are impossible in the adult. Heteroplastic transplantations between adult or nearly adult anurans succeed only with culty (W. Schultz[1]; C. E. Hadley), though it is possible to improve them by posing a period of *in vitro* culture between removal from the donor and imtation into or on to the host (R. Erdmann). Cotronei and his colleagues devoted a considerable amount of work to analysing the reasons for the mpatibility between anurans and urodeles, and have established the curious that anuran transplants into urodeles take better than urodele transplants anurans (Perri[1]; Cotronei & Perri; Spirito[1]; Cotronei & Spirito; Cotronei & reschi). They are of the opinion that where embryonic transplants offer culty, yolk absorption is responsible.

s regards the ontogenetic series, we have the numerous facts due to Idzumi; chewski and others (reviewed in *CE*, p. 1444), which show that the immunoal character of organisms varies during their development.* Antigens come being at successive points in time.† Here should be quoted the pioneer riments of Braus[2] in 1906. He found that adult amphibia (*Bombinator ypus*) contain an antigen which would produce a precipitin in rabbit blood, that this precipitin could not be formed by frog embryos and tadpoles of the species. They therefore do not contain the specific protein characteristic ler stages. Recently a complete piece of work of this kind has been carried n the egg, embryo, larva and adult of the bee by Avrech & Heronimus, with ar conclusions.

rallels to this may be found in the literature on chorio-allantoic grafting. work of Murphy and his collaborators (reviewed in *CE*, p. 1454) showed that ed rat tumours may grow well on the chorio-allantoic membrane of the , but that towards the 18th day of development some change takes place‡ e host which leads to the leucocytic infiltration and death of the piece of on the chorio-allantois. That other mammalian tissues and mammalian yos may grow and develop in this situation has already been made clear

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Section 1·5 above. The classical work of Nuttall in 1904 on immunological differences een animal species is now being extended on precise genetical lines (Irwin; Irwin & Cole; , Cole & Gordon). Cf. all the work on blood groups in man.

the recent work of Baumann[2]; Baumann & Witebsky; Witebsky & Szepsenwol.

rphy traced this effect in part to the spleen; an organ which has since been confirmed to powerful inhibiting effect on the growth of transplanted tumours (Chaletzkaia[1, 2]). There eed, a large literature on the anti-carcinogenic action of the spleen.

(p. 336).* That the embryonic tissues of birds have little specificity has a appeared from many grafting experiments (p. 333). C. J. Sandstrom[1] has inve gated this point further. He found that only young undifferentiated duck kidr tissue would grow in chick eggs as a chorio-allantoic graft. Young duck metaneph tissue which would do this, however, would not heal properly when implant into hatched chicks, forming large tumour-like nodules instead. Sandstrom J. T. Kauer then found that minced duck kidney tissue from embryos of 13 da incubation would grow well on the chick chorio-allantois, while that from 21- days' incubation would not. Transplantation of minced duck kidney tissue fro ducks 1 week after hatching caused the death of the host within 48 hr. The experiments give an unusually clear glimpse into the development of tiss species-specificity. Not all tissues would do so; thus duck embryo.cartilag which did not become vascularised, developed on the chick chorio-allanto without causing any disturbances (Sandstrom & G. L. Kauer).

In a series of papers Waterman[3] has compared the development of rat and rab embryos on the chick chorio-allantois and implanted into the omentum or und the kidney capsule of adults of their own or related species. He concludes th "embryonic tissue of an animal of another class is apparently more tolerant tha adult tissue of a closely related host". Perhaps the most surprising exemplifica tion of this is the transplantations of Oppenheimer[4] already referred to (p. 17 in which half-blastulae of *Danio rerio* (the zebra fish) were implanted into th blastocoele cavity of amphibian eggs (*Triturus torosus*). The fish tissue develope wherever located, and its mesenchyme cells even mingled with those of th amphibian to form a composite tissue. Neural induction of course occurre as has been described. Oppenheimer[8] later showed that though fish an amphibian epidermis and cartilage may grow into a continuum, fish grafts ar never vascularised.

If embryonic tissues have so little species-specificity it should follow that the raw materials could be exchanged with little injury. How far this could be pushe is at present unknown, but there are statements that bird embryos can use th yolk and white of other bird species (*CE*, p. 1444) and this has recently bee confirmed by Ermakov[2], who replaced part of the chick's egg-white with that o the pigeon, and by T. Li, who interchanged the albumens of the gastropod *Limnaea* and *Agriolimax*. Moreover, the growth-promoting factor in embry extract (see p. 628) is not species-specific, as Kiaer[1] and others have shown.

It is generally accepted that the substances responsible for tissue incompati bility, when it appears, are protein in nature. An indication that this may no always be so is provided by the curious finding of Twitty & Elliott, in th transplantations already referred to between *Amblystoma tigrinum* and *Tritur torosus*, that *Triturus* eyes grafted into *Amblystoma* cause complete paralysis o the hosts, from which they do not recover until a few days before they begin t feed. Development is otherwise entirely normal, both for graft and hos abiosis experiments showed that the *Amblystoma* paralysis continues until the nplete loss of yolk from the *Triturus* partner (Twitty & Johnson). Correndingly, it was found that *Triturus* egg extracts cause paralysis when injected *Amblystoma*, but not extracts of the larvae. Adult female blood, doubtless ause of the conveyance of the reserve materials to the ovaries, was also ve in this respect. The substance is not active when administered by the uth; it is destroyed by boiling and not soluble in organic solvents. When npared with the active principles from the skin glands of certain toads formulae on p. 251), it was found to have a quite distinct physiological action, not on the heart rate, but, as was ascertained by ingenious transplantation eriments, exclusively upon the nervous system (Twitty[4]). Nevertheless, this does not resemble the common effects of tissue incompatibility, for the eye other tissues heal well in these interspecific transplantations, and it may hat a non-protein substance is involved.

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* Moreover, the chorio-allantoic membrane is an excellent site for the cultivation of virus (according to a mass of work summarised in Burnet's monograph), though many of these will no grow in the adult fowl.

Vhat may be another example of this is the case described by Brinley & kins where in transplantations of heart, but not of eye, liver or spleen, between bryos of two different species of *Fundulus*, the host's heart ceases after a time beat and death ensues. The fact that the heart of the host stops beating first kes one suspect some incompatible influence of a humoral character. Yet ther toxin discovered by transplantations is described by Humphrey & Burns.

Ve have seen, then, that embryonic compatibility and organiser influences scend genetic boundaries, but that the response is always conditioned by the etic character of the reacting tissue. What else does the nucleus do in early elopment, and how are the characters of the phenotype brought into being he embryo?

·64. The role of the nucleus in development

o-day we are accustomed to think of the nucleus as the repository of the mosomes and genes which during embryonic life develop the individual acteristics of the completed organism, giving it blue fur instead of white, ong bristles instead of short ones. But how far the nuclear equipment is onsible for the specific, generic, or even class characters of the organism is lly as yet settled. The attempt to throw light on this has led to the very large unt of work on cross-fertilisation (hybridisation), but it has of course been usly hampered by the impossibility of obtaining crosses from widely different nisms, and the tendency to weakness and early death of crosses from even y nearly related organisms. Lack of space will prevent any full consideration his literature here, except in so far as it assists us to envisage the processes of ryonic development. Moreover, the uncertainty as to exactly what the leus is doing in the earliest stages of embryonic development has led to eriments in which every kind of combination and permutation of gametic zygotic material has been investigated. In general it may be said that the d of recent work has been to show that what was not thought viable before, actually be so, a fact which gives much hope of further similar advances in future.

The terminology is here a little complicated. Without going into the histor the various terms, we shall follow in the main Fankhauser's recent review[5]. Table and Fig. 205 summarise the principal possible relationships between nucle and cytoplasm in early development. True hybridisation involves fertilisation foreign sperm followed by nuclear fusion (1), but foreign sperm may also used to fertilise an egg the nucleus of which has previously been put out action (4a), or a fragment of an egg containing a normal egg nucleus (5a), o fragment of an egg not containing the egg nucleus (6a), or, finally, foreign spe may be made to fertilise an egg, and the egg nucleus, together with a cert amount of cytoplasm, may be removed before nuclear fusion has occurred (7 Parthenogenetic fertilisation may be carried out, either on the normal egg (or on an egg fragment containing the egg nucleus (8), or even on an egg fragme containing no nucleus at all (9). An egg may develop while carrying only spe nucleus and its derivative nuclei, either because the egg nucleus has been p out of action beforehand (4), or because a fragment of the egg not containing t egg nucleus has been fertilised (6). This latter case (with the special modificati of the removal of as little cytoplasm as possible with the egg nucleus) has afforde as we shall see, one of the most fruitful ways of penetrating into the question the role of the nucleus in early development.

An exhaustive survey of the literature of these many types of developme cannot here be attempted, but an approach to it will be facilitated through t papers quoted and reviews such as that of P. Hertwig[2] on hybridisation in gener It will be convenient to take the different cases in the order in which they occ in the table, and in Fig. 205.

The general characteristic of all the haploid types is the abnormality of the development, and their tendency to early death. Those embryos that surviv hatching form larvae showing stunted growth, delayed differentiation, reduce motility and reactivity to stimuli, and general oedematous swelling of the bod The detailed exploration of these anomalies should throw a great deal of lig on the normal mechanisms which have here been interfered with.

The most obvious way of retaining the haploid condition of the unfertilis egg is by *Parthenogenesis*, thus entirely excluding the sperm chromatin. T action of the spermatozoon may be partly replaced by pricking with a gla needle, the action of chemicals, etc. which "activate" the egg. The reviews Parmenter, Peacock and Rostand on parthenogenesis should be consulted her In the invertebrates it occurs naturally, and just as polyembryony has becom fixed as a species characteristic in many parasitic hymenoptera (cf. p. 228), parthenogenesis regularly occurs in many hymenoptera and coccids (see t review of Schrader & Schrader.* In vertebrates, only the faintest traces spontaneous parthenogenesis have been met with, but experimental parthen genesis has been achieved even for mammals (Pincus & Shapiro).

After the successes of J. Loeb[3] in 1899 with echinoderm eggs, Bataillon attacke the problem for the amphibia at the turn of the century—references to th older literature will be found in Loeb's review. It is now known that kinds of agents will bring about parthenogenetic activation in amphibian egg traumatism (pricking), hypertonic solutions, heating followed by cooling,

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* For the chemical parthenogenesis of silkworm eggs, see p. 583.

versa, exposure to electric induction shocks, organic solvents, etc. But a inction is made between "activation", which determines a certain number he preliminary phenomena of development, and "regulation", which permits avage and further development. The treatments described above only lead to tivation", i.e. they cause orientation of the egg, the appearance of the second ar body, the appearance of the membrane, the onset of unfertilisability, and formation of the grey crescent. In order to add the "regulation" factor it is olutely necessary to inject into the egg an active substance present in the od and tissue fluids of the adult. It is easy to see how erratic the earlier results e, for a slight contamination of the exterior of the egg would lead to the belief full parthenogenetic development had been obtained by pricking. This covery was due to Guyer's experiments of 1907, and though at first Bataillon[1] osed Guyer's views, he later demonstrated himself[2] that absolutely clean eggs uld never develop after pricking alone (1911).*

he blood factor was soon found to be quite non-species-specific among vertebrates† (Bataillon[2]) and to be present in all organ extracts and juices gucki[1]). Cell-free extracts of blastulae and gastrulae are as effective as blood, but similar extracts of unfertilised eggs or two-cell stages (Bogucki[1])—a remarkable allel to the liberation of the organiser substance (cf. pp. 174 ff.). The factor nly contained in nucleated cells; thus the erythrocytes of mammalian blood ne are inactive, while the leucocytes are active, but the nucleated erythrocytes auropsid or amphibian blood possess the factor (Bataillon[4]). The factor seems e present in some invertebrate tissues; thus positive results were obtained h the testes of *Lumbricus* and the hermaphrodite gland of *Helix* but not with stacean blood or sperm or *Dytiscus* sperm (Bataillon[4]), nor with any protozoan, terial, or plant material (Rostand). There are variations in the state of activity the factor. Molluscan testis tissue extracts allow of cleavage but never of trulation, while horse leucocytes allow gastrulation but not usually hatching, with bird leucocytes large numbers of tadpoles may be produced.

he blood factor is not stable to boiling, but is destroyed at about 55° C. sele[1]), although the motility of amphibian spermatozoa ceases already at 37°. stable to desiccation over sulphuric acid (Rostand), but it is destroyed by the on of denaturing agents such as organic solvents; hence it is probably a protein perhaps an enzyme.‡ The molecule cannot, however, be very large, as it passed ugh collodion ultra-filters in the experiments of Parat. No successful effects so far been obtained by injecting into the egg solutions of purified enzymes as pepsin, rennin, etc. A nuclein prepared from erythrocytes by Bogucki[1] was tive, and many other substances similarly injected by Rostand gave no results.

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he delay in the acceptance of Guyer's facts was perhaps due to his own rather fantastic theory eucocyte proliferation within the stimulated egg.

ence the paradoxical result reached by Bataillon[3], that much better development took place a *Bufo* egg parthenogenetically fertilised by injection of frog blood, than from one properly ised by frog sperm, including nuclear fusion. Inadequate nuclear fusion (hybridisation) d not in this case equal the haploid parthenogenetic state.

ut a very short heating to 75°, which coagulated most of the protein in a blood extract, did according to Einsele[1], inactivate it.

Table 24. *Terminology of possible relationships between nuclei and cytoplasm in development*

			Term employed	
(1)	Egg fertilised by foreign sperm	Diploid nucleus divides	Hybridisation (cross-fertilisation)	D
(2)	Egg fertilised by artificial means	Egg nucleus alone divides	Parthenogenesis*	H
(3)	Egg fertilised by sperm so treated that nuclear fusion cannot ensue	Egg nucleus alone divides	Gynogenesis	H
(3*a*)	Egg fertilised by foreign sperm incapable of nuclear fusion	Egg nucleus alone divides	Hybrid gynogenesis ("false hybrids")	H
(4)	Egg, the nucleus of which has been so treated that nuclear fusion cannot ensue, fertilised by normal sperm	Sperm nucleus alone divides	Androgenesis	H
(4*a*)	The same, with foreign sperm	Sperm nucleus alone divides	Hybrid androgenesis	H
(5)	Fragment of an egg, containing the egg nucleus, fertilised by sperm	Diploid nucleus divides	Diploid merogony	D
(5*a*)	The same, with foreign sperm	Diploid nucleus divides	Hybrid diploid merogony	D
(6)	Fragment of an egg, not containing the egg nucleus, fertilised by sperm	Sperm nucleus alone divides	Andro-merogony†	H
(6*a*)	The same, with foreign sperm	Sperm nucleus alone divides	Hybrid andro-merogony†	H
(7)	Fragment of an egg, containing the egg nucleus, removed from an egg fertilised by sperm, before nuclear fusion	Egg nucleus alone divides	Gyno-merogony	H
(7*a*)	The same, with foreign sperm	Egg nucleus alone divides	Hybrid gyno-merogony	H
(8)	The same, fertilised by artificial means	Egg nucleus alone divides	Parthenogenetic gyno-merogony	H
(9)	Fragment of an egg, not containing the egg nucleus, fertilised‡ by artificial means	No nucleus present at all	Parthenogenetic merogony	NN

* Parthenogenesis in the invertebrates which is diploid because the reduction division has been omitted is not considered here (see Waddington[22], p. 59).

† If the nucleus is removed with but a minimum of cytoplasm, this approximates to (4) and (4*a*), save that no remains of the egg nucleus are present.

‡ Many writers use the term "activated" for some of the above cases; I reserve it, as Bataillon[4] did, for those processes which can be made to happen in the absence of the "blood factor". Thus fertilisation = activation + regulation.

N.B. The letters in the right-hand column indicate the resulting situation; D = diploid, H = haploid, NN = non-nuclear.

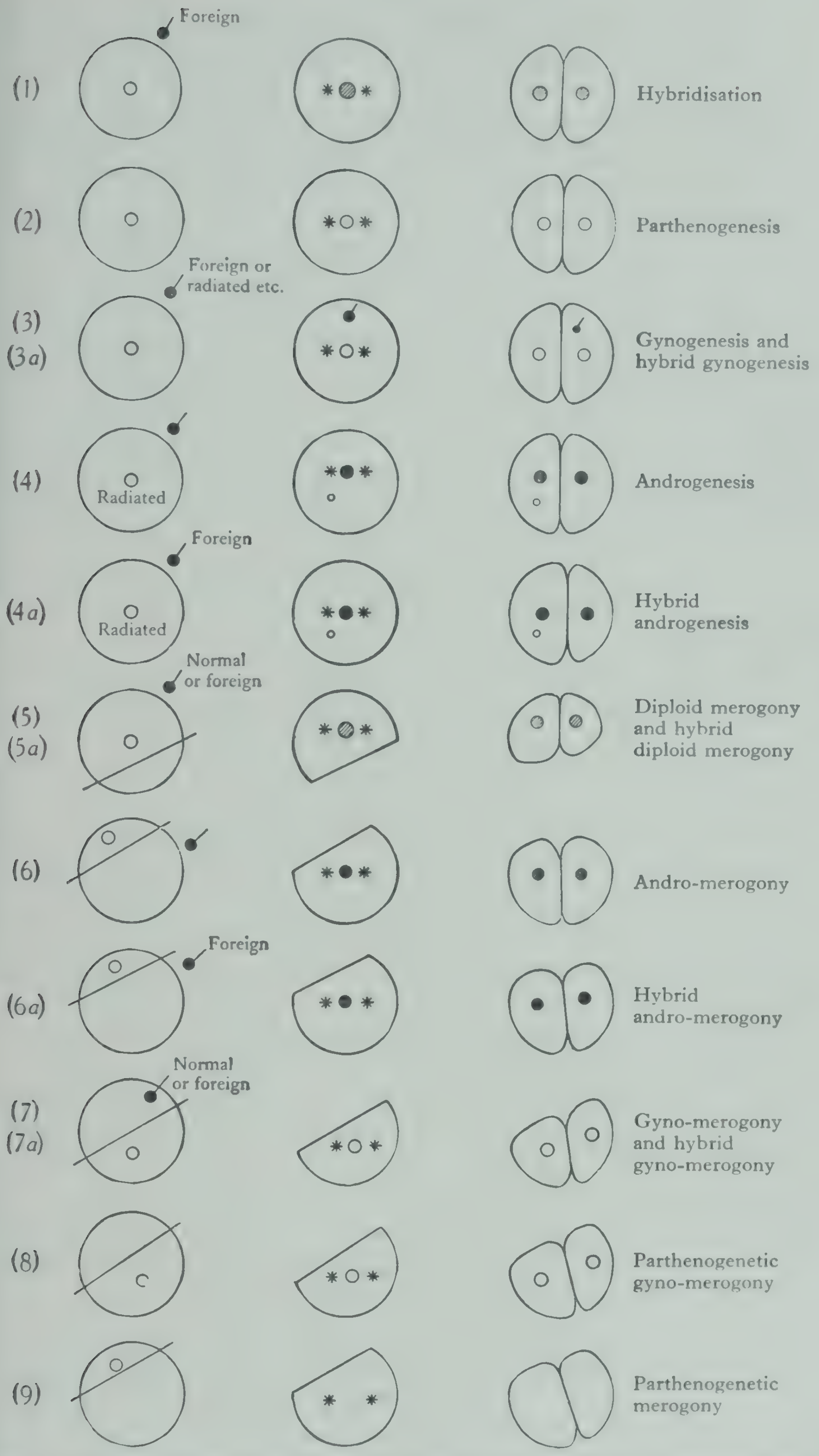
Foreign
(1)
Hybridisation
(2)
Parthenogenesis
Foreign or radiated etc.
(3)
(3a)
Gynogenesis and hybrid gynogenesis
(4)
Radiated
Androgenesis
Foreign
(4a)
Radiated
Hybrid androgenesis
Normal or foreign
(5)
(5a)
Diploid merogony and hybrid diploid merogony
(6)
Andro-merogony
Foreign
(6a)
Hybrid andro-merogony
Normal or foreign
(7)
(7a)
Gyno-merogony and hybrid gyno-merogony
(8)
Parthenogenetic gyno-merogony
(9)
Parthenogenetic merogony

Fig. 205. Possible relationships between nucleus and cytoplasm in development.

In immature eggs, taken from the body-cavity of the female, parthenogen by pricking and injection of the blood factor cannot be brought about successf (Barthélemy).

Bogucki[2] and Rapkine[1] subsequently extended this work to echinoderms showed that parthenogenesis by pricking is possible for echinoderm eggs a if blood or tissue juice is present at the same time, or extracts of embr from the blastula stage onwards but not before. This, of course, is only one many treatments which will give echinoderm parthenogenesis (see p. 214).

The general upshot of all this work is that in normal fertilisation the spe nucleus brings in with it something, apparently an enzyme, which, though pres in all adult cells which have nuclei, is either absent, present in insufficient amou or in some way masked, in the unfertilised egg. Since the isolated acrosome alo was effective, producing haploid fertilisation, Parat thought that the blood fac must normally be present in this extreme "cephalic" cap of the spermatozo How exactly this substance contributes to normal development, or rather, h it permits the normal cleavages, gastrulation, etc. which cannot go on in its absen still remains altogether obscure.

Parthenogenesis in fishes, birds and mammals has not been analysed anythi like as completely as it has in the amphibia; details will be found in the mon graph of Rostand.

We come now to *Gynogenesis*. If spermatozoa, before insemination of t eggs, are treated with moderate doses of radium emanation, *X*-radiation, ultr violet irradiation, or certain chemicals such as trypaflavin, their ability to ent the egg will not be affected, but no fusion of the sperm nucleus with the e nucleus will follow. The cytology and embryology of the effects of this have be studied by the Hertwigs (O. Hertwig[3]; G. Hertwig[1]; P. Hertwig[1]) and by Dalc and his collaborators (Dalcq & Simon; Simon[1], etc.). The outstanding fact interest to us is that the gynogenetic eggs usually suffer a characteristic imped ment to gastrulation, though if the irradiation or other treatment of the sper has been very short, they may successfully pass through gastrulation and gi free-swimming haploid larvae which may live as long as a fortnight. As Ru has shown, development is never affected before gastrulation, however severe the sperm have been irradiated. The more we know about the metabolism of t gastrula, the more profitable it will be to analyse the nature of the paralysed sta (delayed effect) into which it enters after this pre-fertilisation treatment of t sperm.

Gynogenesis can also be brought about by hybridisation between differe species of frogs and toads, in which the foreign sperm activates the egg, but fa to fuse with the egg nucleus, and plays no further part in development (G. Hertwig S. Ch'ou[1]; Bataillon & S. Ch'ou; Rugh & Exner). Such embryos are call "false hybrids"; a typical case is Hertwig's *Rana esculenta* ♀ × *Bufo viridis* They can be definitely distinguished from true hybrids if the sperm is previous irradiated; this will interfere with development if nuclear fusion usually tak place because it will prevent it, but if, as in the case of the false hybrid, it do not take place, no additional troubles in development will appear.

all these cases, regulation from haploidy to diploidy may occur, either by ision of chromosomes not followed by cell-division or by a resumption of hromatin material of the second polar body.*

istly, gynogenesis may be brought about by treatment of the egg with cold. ses of *Bufo vulgaris* ♀ × *Rana temporaria* ♂ normally fail to gastrulate, but and was able to prevent nuclear fusion by exposing the eggs to low tempera- ; in this way they developed "parthenogenetically" under the influence of gg nucleus only and gave free-swimming larvae.

Androgenesis, the egg develops solely under the influence of the sperm eus. Treatment of unfertilised amphibian eggs with radium seems to have ively little effect on the cytoplasm, but it puts the egg nucleus out of action, so prevents nuclear fusion (G. Hertwig[1]; P. Hertwig[2]; Dalcq[4]). Or the egg eus may be removed with a minute pipette before fertilisation (Curry; lor). These androgenetic eggs suffer a marked delay at gastrulation, but iding their nuclei have the exact haploid chromosome number, they frequently inue to develop and up to the present time have been cultivated as long as stage of fore-limb-bud development (Kaylor; Porter).

645. Merogons and chimaerae.

far we have been considering only those cases in which the main mass of gg cytoplasm participates in the development of the organism. Development gg fragments is called *Merogony*. The simplest case is that in which the fusion e egg and sperm nuclei is prevented by ligaturing the egg into two parts. was first accomplished for the newt, *Triton taeniatus*, by Spemann[7] in 1914; part containing the sperm nucleus will develop into a haploid larva. It was investigated by Baltzer[1] and by Fankhauser[2]. In this method not only is the iber of chromosomes reduced to one-half but the amount of egg cytoplasm rrespondingly reduced. The fact that such embryos become abnormal and lisposes, therefore, of G. Hertwig's suggestion that the cause of the diffi- s of haploid embryos lies in the excessive amount of yolk which they contain. ed, an exactly opposite explanation was advanced by Ch'ou. In his gyno- tic embryos already mentioned, it was observed that yolk utilisation proceeded faster than in the diploid controls; his suggestion therefore was that as the nuclear surface was greater owing to the smaller volume of each nucleus, bnormally high interaction rate between nucleus and cytoplasm led to an rmally high metabolic rate, sufficient to kill the animals. So far none of theories has been put to experimental test with accurate methods. Another r in the haploid "syndrome" is the oedema which, as Dalcq & Simon have ited out, cannot be due to insufficiencies of the earliest excretory organs as , since it begins already in the gastrula stage. It must be remembered, ever, that gastrular and neurular oedema is quite commonly met with in the vation of diploid embryos. A genetical interpretation of the haploid drome" has been suggested by Darlington, who thinks that all haploid

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parently it may go a great deal further. Kawamura describes polyploid frog larvae obtained rthenogenesis—mostly viable—but as weak as haploid ones if hexaploid or over.

embryos may have at least one lethal factor hidden in their chromosom Unmasked by the absence of its normal allele, this will kill the embryo soo or later. Unfortunately such an explanation will not account for the healt behaviour of haploid embryos which have regulated to diploid by a nucl division not followed by a cell-division (Parmenter). These should now homozygous for the lethal factor, and hence should be less instead of mo healthy than the haploid embryos. So King & Slifer have shown that parther genetic grasshoppers are not viable unless regulation to diploidy takes pla One fact at any rate is undoubted, namely that the high rate of mortality merogonic embryos during gastrulation is associated with the frequent occurren of cells with a sub-haploid or otherwise unbalanced chromosome numb (Fankhauser[3]). What exactly this means in terms of physiology and biochemis remains to be determined.

If an egg is divided into two after fertilisation but before nuclear fusi the part containing the egg nucleus alone develops much less well than t containing the sperm nucleus alone, generally passing through only a f cleavages (Woerdeman[5], on echinoderms), but Fankhauser[4] has recently obtain very abnormal blastulae from such fragments in amphibia (*Gyno-merogons*). Spemann's original work, the fragment of the egg other than that containi the sperm nucleus alone contained an egg nucleus fused with a sperm nucle since in the species with which he was working polyspermy normally occurs

The greatest advances in our knowledge of the role of the nucleus in ea development have arisen from the study of *Hybrid andro-merogons* by Baltz Hadorn and their collaborators.* Andro-merogons were first obtained by Bove in 1889 on sea-urchin eggs, and many subsequent workers have studied them the hope of deciding what specific characters, if any, are carried by the cytoplasm, but in general they do not live long enough to permit of the ex determination of the species characters. Spemann's experiment above mention was extended in 1920 by Baltzer[1] to include cross-fertilisation and the study the resulting hybrid andro-merogons. Such combinations are written with the sign of the egg in brackets to show that the egg nucleus has been removed, th

Triton taeniatus (♀) × *Triton palmatus* ♂.

It was soon established that these embryos would pass through the gastrulat stages without difficulty, but died in a characteristic way as neurulae. Althou many tissues and organs (such as epidermis, neural tube, and notochord) remai healthy, the head mesoderm lying between notochord and foregut began degenerate at a certain stage, the appearance of pycnotic nuclei preluding co plete histolysis. As a result of the spread of this degeneration to the rest of body, death of the embryos followed.

The problem arose, therefore, of analysing a little more deeply the causes this "disease", and the first contribution of Hadorn[1] was devoted to showing t tissues other than presumptive head mesoderm, isolated from a haploid emb of the cross *T. palmatus* (♀) × *T. cristatus* ♂, would, when transplanted int

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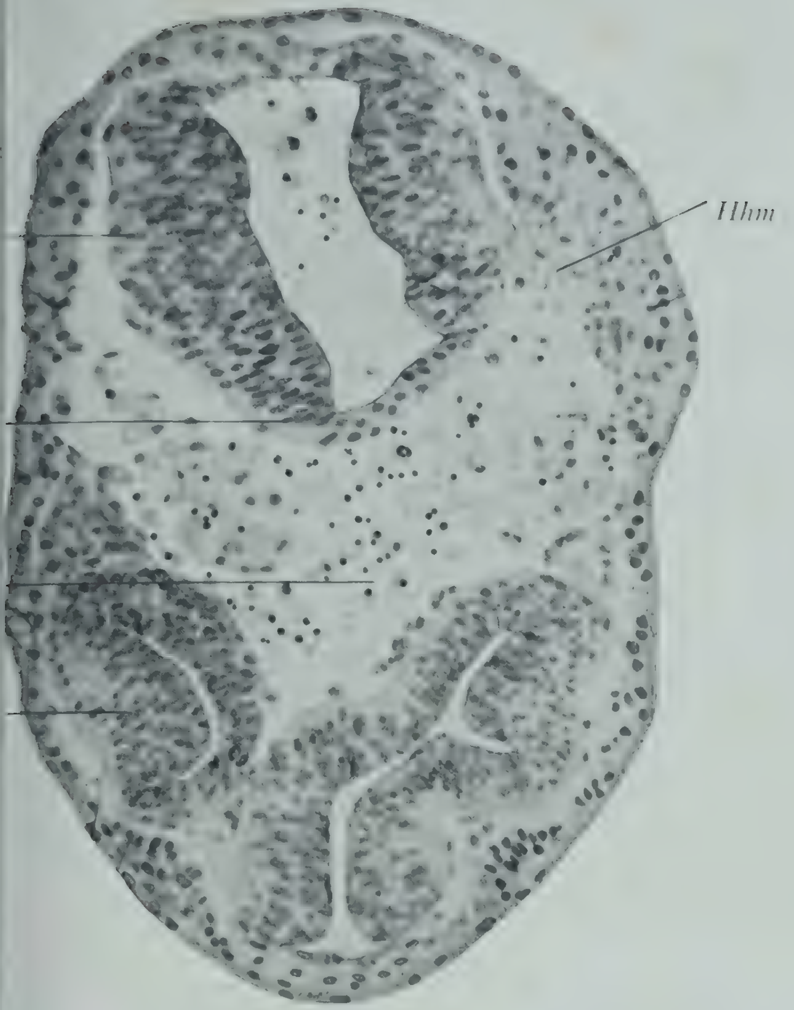
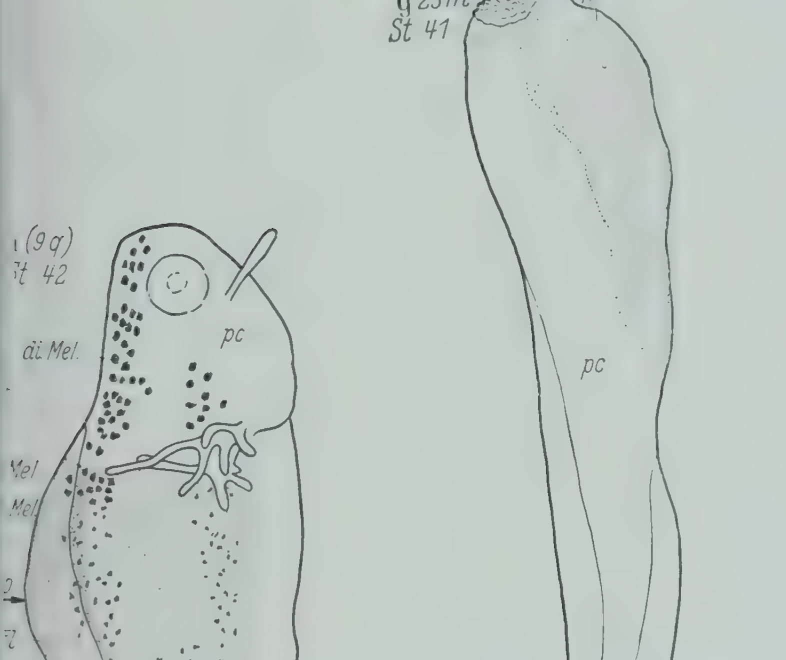
* Reviews by Baltzer[3, 4, 6].



Spontaneous disintegration of head-organiser mesoderm of
ıdro-merogon even when surrounded by normal tissue. *Hb*,
ion of brain; *Gb*, graft portion of brain; *Hhm*, healthy host
n; *Ghm*, degenerating graft mesoderm; *Hoc*, host eye.



Chimaerae in which hybrid andro-merogon tissue (stippled)
d with normal diploid tissue (white). A. The normal anterior
duce a fairly normal trunk but not a normal tail in the haploid
The normal posterior end forms a normal tail, but the haploid
d can do nothing. *Fl*, fin; *ha.Mel.*, haploid melanophores;
ploid melanophores; *e.Mel.*, diploid melanophores migrated
ploid tissue; *Bl.*, epidermal blister in tail-region; *H*, func-
oid heart.

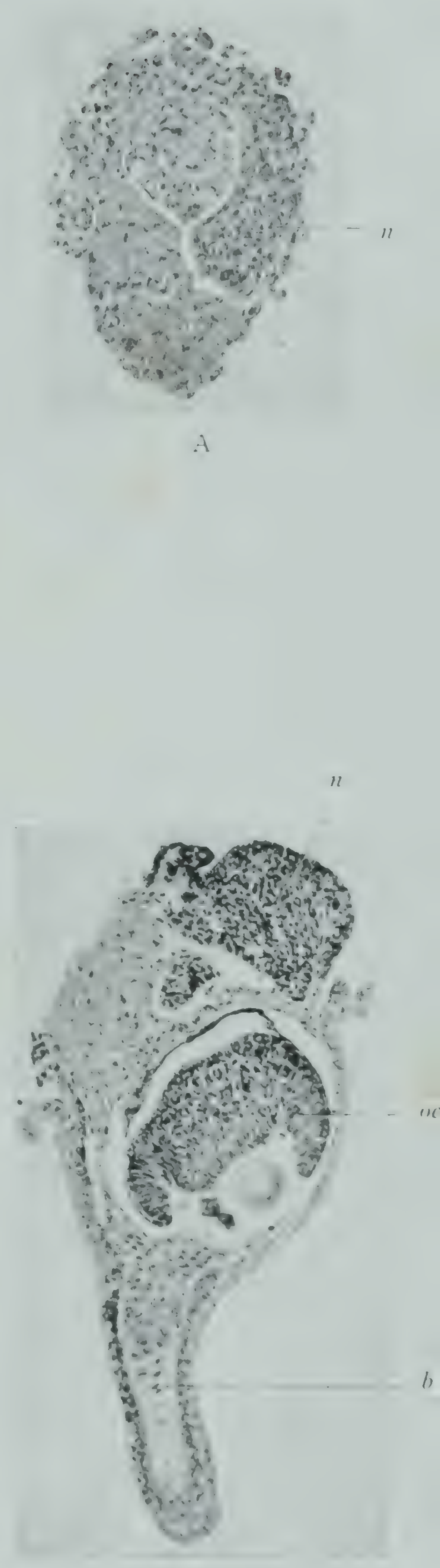


Fig. 207. Differences between differentiations in explants. A. Material from a hybrid andro-merogon makes only chaotic neural tissue (*n*). B. Material from a hybrid diploid embryo makes brain (*n*), eye-cup (*oc*), lens, and balancer (*b*).

nal diploid embryo, develop perfectly normally. Thus presumptive epidermis t to form normal skin, ear-vesicles, and blood-cells; presumptive neural tissue t to form normal eye-cups and brain; even presumptive trunk somite material normal somites. Only presumptive head mesoderm persisted in degenerating ts new environment. Fig. 206, taken from Hadorn's paper, shows the dis- gration of the head-organiser tissue. These facts demonstrated two things, that there is a specific "haploid disease" of head mesoderm, which trans- ntation into a normal host cannot cure, and secondly that the remaining tissues ne haploid embryo are perfectly capable of indefinitely long life and differentia- if they are removed from the influence of the disintegrating head mesoderm.*

low far are the neighbouring tissues of the host responsible for this high erentiation capacity of the haploid tissue? This was answered by Hadorn[2], , adopting the explantation methods of Holtfreter (see p. 157), cultivated ous parts of these andro-merogonic haploid gastrulae *in vitro*. In this way as possible to show that parts other than presumptive head mesoderm would much longer than if they had remained in the andro-merogon itself. In some s, such as presumptive notochord, they would differentiate histologically fully. But in no case was the differentiation seen in these explants as good nat, either of such parts transplanted into a diploid host, or of parallel explants n a non-hybrid andro-merogon, or of parallel explants from a hybrid but merogonic embryo. Thus Fig. 207 shows that while the hybrid andro- ogonic material made only chaotic neural tissue in explant, the hybrid diploid ue formed a good eye-cup with lens, and a balancer. Thus not haploidy uch, nor hybridisation as such, could be held responsible for the low self- erentiation capacity of the tissue, but presumably the mixture of both factors. l what was remarkable was that this low differentiation capacity could be so ch enhanced by *contiguity* with normal diploid embryonic tissues.

n order to get further light on the hypothetical "histogenetic substances" h it seemed necessary to postulate to explain this effect (*entwicklungs- rnde Hilfe*), Hadorn[3] next combined halves of hybrid andro-merogonic ryos with halves of normal diploid embryos to form various beautiful types chimaerae". The results may be summarised by saying that provided a nal head-organiser is present, the rest of the tissues will develop normally, that the influence of the normal diploid tissues seems to spread out in a kind radient into the others. We may first compare the difference between an ryo of which the anterior half is haploid tissue and one of which the anterior is diploid tissue. The former, inhibited by its degenerating head mesoderm, formed a tail and trunk structures but nothing else; the latter has produced ell-formed brain, eye, and balancer, besides fairly normal trunk structures . 208). The influence favouring differentiation which seems to radiate from diploid tissue is well seen in Fig. 209. This chimaera had a diploid anterior

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e are reminded here of the specific mesoderm-inhibitory substance in malt extract discovered leaton and carefully investigated by Medawar[1]. It appears to be of carbohydrate nature and ssess aldehydic groups (see p. 629), and it has no effect at all on ectoderm and epithelia. It, or thing very like it, can be extracted from adult liver (Brues, Jackson & Aub). Medawar[2] ts it inhibits the cell-movements at ana-telophase.

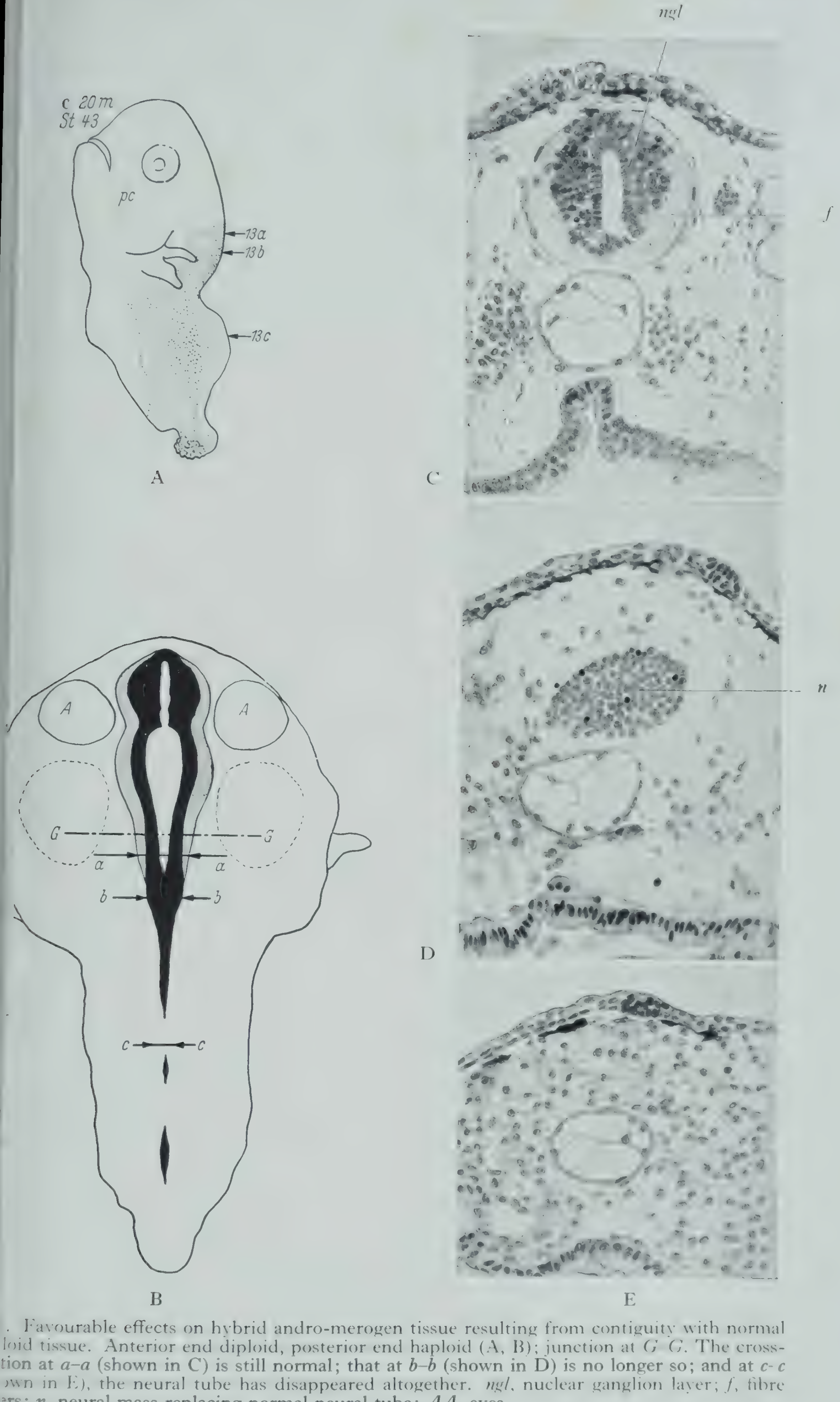
end and a haploid posterior end. The diagram shows that some distance caudall the established junction between the two types of tissue, the form of the ne tube was entirely normal. Still further posteriorly, however, it became v abnormal and finally disappeared altogether.

Two other types of chimaerae were made, one in which sagittal halves w combined, in which case considerable lengths of normal neural tube tissue w formed from the haploid tissue; and another in which diploid organiser mate was enveloped in haploid presumptive ectoderm. These latter developed qu normally, giving larvae which even passed through metamorphosis, althou their skin was purely haploid. Needless to say, the reverse combination g nothing except that an eye-cup was induced, which degenerated before it h had time to form properly.

Subsequent cases proved even more difficult to analyse than the *Tri* *palmatus* (♀)×*T. cristatus* ♂ andro-merogons of Hadorn. The cross *Tri* *alpestris* (♀)×*T. palmatus* ♂ was studied by de Roche. Here the andro-merogo haploid embryos develop to the closed neural tube stage before dying, but special area of degeneration is to be found in them, such as the head mesode previously described. All the tissues fail at the same time. But on the other ha transplantation into normal diploid *palmatus* gastrulae showed that every part the haploid embryos, including the head mesoderm, possesses full powers differentiation in the host. A similar lack of specific disease centre was found the work of Baltzer, Schönmann, Lüthi & Boehringer on crosses of less simil animals, e.g. *Triton palmatus* (♀)×*Salamandra maculosa* ♂. The true hybrid cro here always perished in the gastrula stage, and measurements of the number abnormal nuclei showed a wave of these in the late blastula preceding the rap increase in the number of degenerated nuclei which leads to death (Fig. 21 Schönmann). The andro-merogonic cross fared still worse, seldom surpassin the middle blastula stage owing to the general nuclear disintegration (Boehringer Transplantations of blastula roof from the andro-merogon showed no capaci for further development, but those from the true hybrid cross, though normal doomed to die in the gastrula stage, were found to be capable, in a normal diplo *palmatus* host, of every imaginable kind of normal differentiation (Lüthi). some way or other the lethal tissue can be "vitalised" by a "histogenetic stimulus from the normal host.

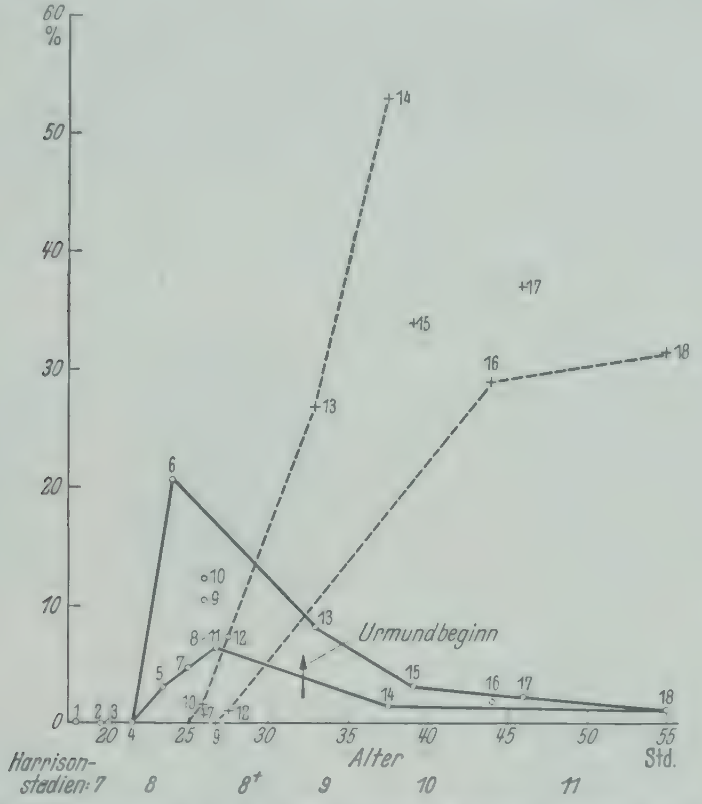
Effects quite analogous to those just described were seen by Strasburger crosses of the ladybird beetles *Epilachna chrysomelina* × *E. capensis*. During blast derm formation the nuclei degenerate and the embryo dies.

The whole subject has been well summarised by Baltzer & de Roche. Apa from Hadorn's disintegration centre in the head mesoderm, other differentiatio inhibiting factors must exist in hybrid andro-merogons, and in some cases, we have seen, they arise in the absence of any disintegration centre. The inhibition, however, is reversible by contact with normal tissue; that of th disintegration centre is not. Such a reversibility (cf. Fig. 209) is reminiscent homoiogenetic induction (cf. p. 154). Whether the inhibition affects mainly th power of inductors or the competence of tissues to react to them is not yet know


ngl
c 20m
St 43
pc
13a
13b
13c
A
A
A
G
G
a
a
b
b
c
c
B
C
f
n
D
E

. Favourable effects on hybrid andro-merogen tissue resulting from contiguity with normal
loid tissue. Anterior end diploid, posterior end haploid (A, B); junction at *G G*. The cross-
tion at *a–a* (shown in C) is still normal; that at *b–b* (shown in D) is no longer so; and at *c-c*
own in E), the neural tube has disappeared altogether. *ngl*, nuclear ganglion layer; *f*, fibre
ers; ***n***, neural mass replacing normal neural tube; *AA*, eyes.

in any case this merogonic lethality has an obvious relation to the many cases wn of lethal genes (see below, pp. 365 ff.), where a gene or group of genes mendelising properties brings about abnormal development. In the first e, both types of lethality act at a definite time in development, as in the cases of *yellow* mouse (Kirkham[2]), the *short-tailed* mouse (P. Chesley) and the *creeper* k (Landauer[23]). In the second place, a specific part of the embryo is affected, the *short-tailed* mouse (Chesley) or the hereditary tumours of the fruit-fly wen; Stark). Thirdly, the lethality can be overcome by transplantation of e of the organs to a more favourable environment.



10. Nuclear disorganisation in the late blastula stage preceding death of the gastrula in ybrids of *Triton palmatus* and *Salamandra maculosa*. The zone between the continuous nes shows the percentages of abnormal nuclei and mitoses; that between the dotted lines ows the percentages of pycnotic and degenerated nuclei.

his has been shown in many organisms other than amphibia. It was first ed for sphingid moths (Bytinski-Salz[1]; Bytinski-Salz & Gunther) in 1930. ough the reciprocal cross is healthy, *Celerio euphorbiae* ♀ × *C. gallii* ♂ causes death of all females as pupae. Yet ovaries and wing buds lived on normally transplantation to normal animals.

hrussi[5] carried the matter further by using tissue-culture technique on the ozygous lethal *short-tailed* mouse. Tissues taken from the posterior regions ch an embryo, which normally dies *in utero* on the 10th day of development, d be cultivated *in vitro* for as long as two months and differentiated into lage. Whether this is a reversal of intrinsic differentiation-inhibition or a val from the degenerating influence of a "disintegration centre" is not yet . Since then Hadorn[4] has described a parallel case. *Lethal-giant* fruit-fly e, at first normal, fail to pupate properly, and survive for a time in a transparent oedematous state before they die. During this time the imag discs degenerate completely. In order to see the extent of this lethality, Had transplanted the ovaries (which develop early like the larval organs but wl persist into the adult state) into genetically normal larvae. The ovaries then v found to develop much further than they do in the lethal larvae, even produ eggs, though not reaching full adult development. It was subsequently sh that the mechanism of the delay in pupation in *lethal-giant* larvae is a failur the *corpora allata* or homologous structures to secrete the pupation hormo (Hadorn[5]; Hadorn & Neel; Scharrer & Hadorn; Burtt).

Hadorn's work has been confirmed and extended on similar *Drosophila* let by Medvedev[3], who could obtain normal development of the imaginal b indefinitely by transplantation to normal larvae.

It is interesting to note that in some cases a hybrid embryo may deve relatively far before nemesis overtakes it. Thus Rubaschev has reported on fantastic contortions exhibited by notochord and neural tube in the trout cros *Salmo fario* × *Coregonus lavaretus*, due perhaps to localised delays at gastrulati And in toad crosses, as Montalenti has shown, there may be failures of limb-b formation. This approaches the question of the integration of organs in fore hosts, which we have already discussed (section 2·62). It is interesting, too, that su delayed lethality can be imitated experimentally. When Geigy irradiated *Drosoph* embryos with ultra-violet light, no defects were traceable until the imaginal sta

A good deal of work has been done on merogony and hybridisation in s urchins. The results are complex and reference must be made to the reviews v. Ubisch[6] and Hörstadius[6].

All that now remains to be discussed in this section is *Parthenogen merogony*. That a fragment of an egg containing the egg nucleus should capable of parthenogenetic development is nothing out of the ordinary. in 1907 McClendon succeeded in sucking out the nucleus from an echinode egg and parthenogenetically fertilising the merogon remaining. Cleav (somewhat irregular) proceeded to the early morula stage. Thirty years la E. B. Harvey[6] treated echinoderm eggs in E. N. Harvey's centrifuge-microsc (descriptions; E. B. Harvey[2,3]; E. N. Harvey[3]) so that they fragmented i halves or quarters. The non-nucleate fragments could be parthenogenetica fertilised and would develop as far as free-swimming blastulae.

E. B. Harvey's previous work[5] on centrifuging echinoderm eggs at high sp had shown that although the distribution of the layers of granules, yolk, e varies from species to species, all the fragments retain their fertilisability, w the result that diploid merogons and andro-merogons develop in large numb when the eggs are fertilised with normal sperm. Cross-fertilisation succeeds approximately the same extent in the merogons as in the whole eggs, so that w foreign sperm hybrid diploid merogons and hybrid andro-merogons are p duced. Fig. 211 A shows unfertilised *Arbacia* eggs stratifying and pulling ap as observed in the centrifuge microscope. Below are the red non-nucleate halv at the right one of the red halves is fragmenting to quarters, at the extreme ri the separation of quarters has just taken place. Apparently quite normal plu

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* For an account of this see below, p. 465.

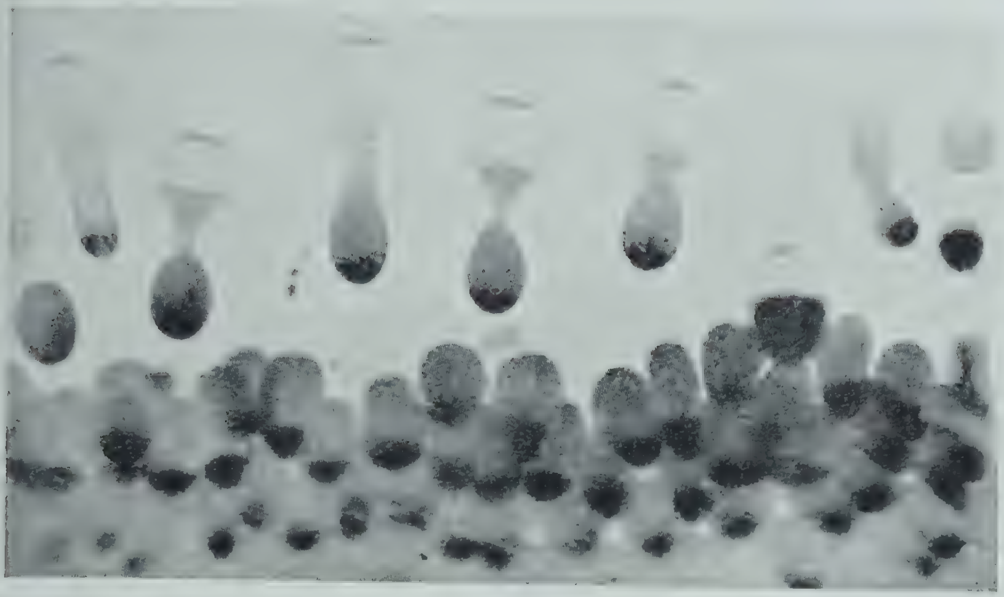


Fig. 211 A. Unfertilised echinoderm eggs stratifying and pulling apart under the influence of strong centrifugal force.

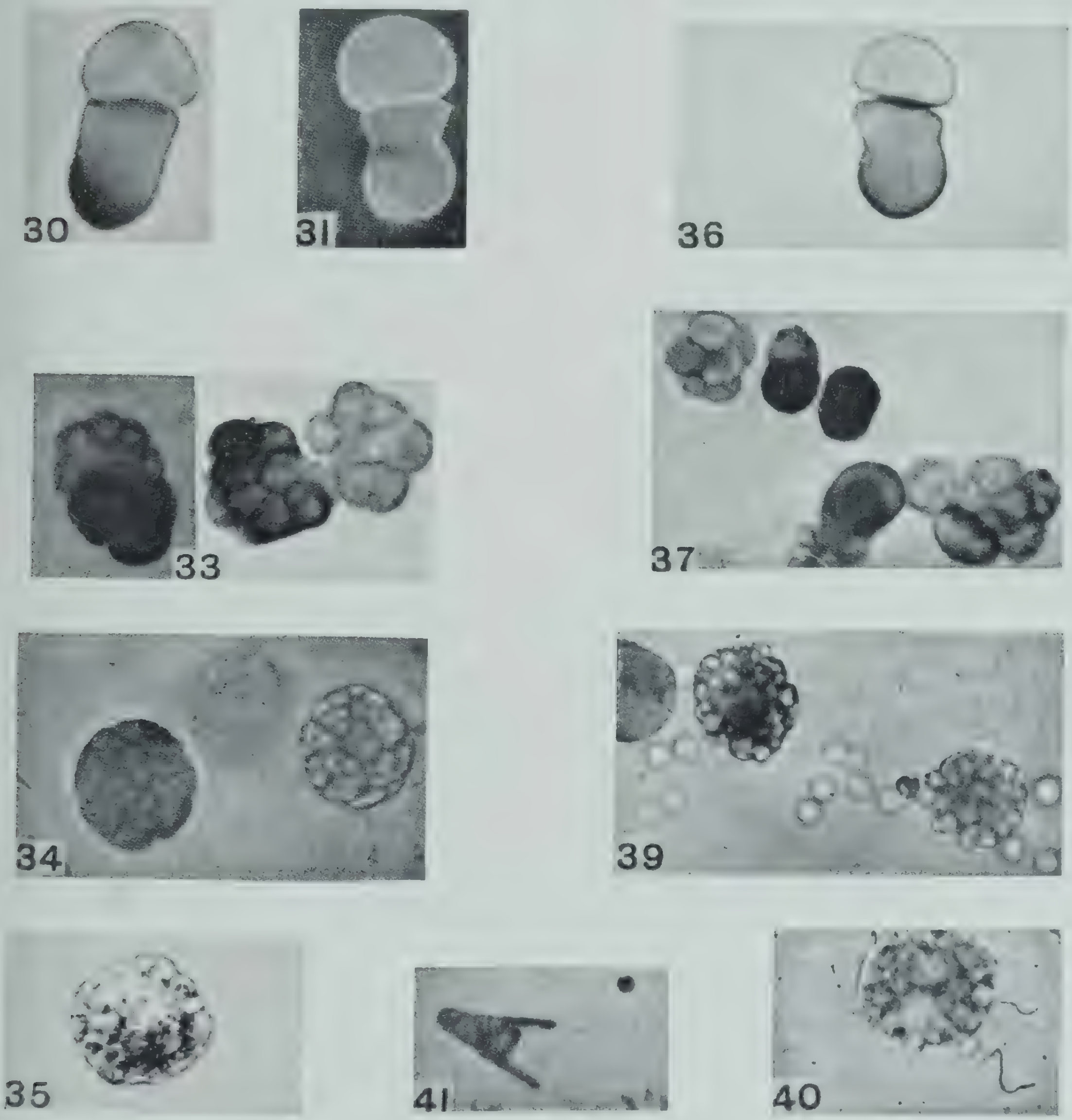
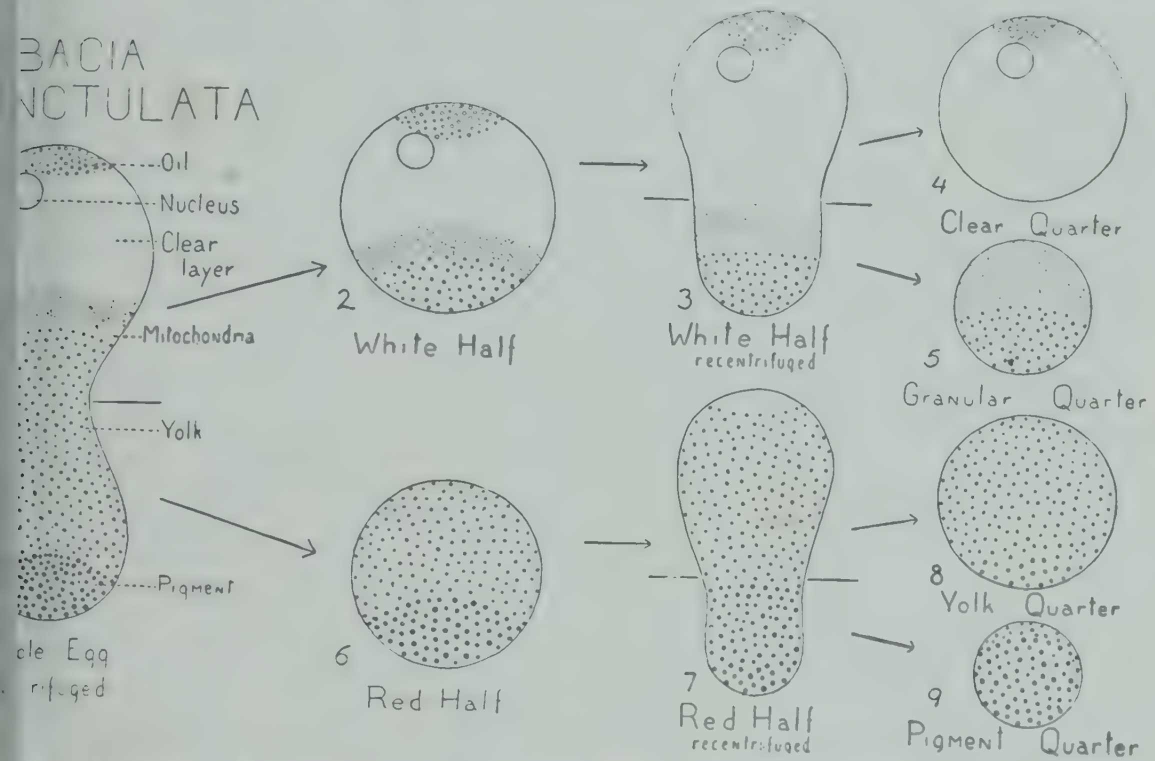


Fig. 212. Development of non-nucleate parthenogenetic merogons as far as the blastula stage in the sea-urchin *Arbacia*.

frequently raised from the andro-merogons, which in some cases (e.g. *erechinus granularis*) developed better than the diploid merogons of the species.

the work on parthenogenetic merogony *Arbacia* eggs were centrifuged into quarters, as follows, centripetally: (1) clear quarter, containing oil-cap, us, and hyaline cytoplasm, (2) granular quarter, containing some yolk, lk quarter, and (4) pigment quarter (Fig. 211 B). The nucleate quarter gave parthenogenetic gyno-merogons, but the granular quarter was difficult to n, so most of the parthenogenetic merogons were produced from the third ourth quarters. Hypertonic salt solutions were used as activating agents. non-nuclear merogons throw off fertilisation membranes, and the successive


BACIA
NCTULATA
Oil
Nucleus
Clear layer
Mitochondria
Yolk
Pigment
cle Egg
r-f-ged
2
White Half
3
White Half
recentrifuged
4
Clear Quarter
5
Granular Quarter
6
Red Half
7
Red Half
recentrifuged
8
Yolk Quarter
9
Pigment Quarter

1 B. Diagram of the stratification of an echinoderm egg and its separation into merogons.

ges are regularly preluded by the appearance of cytasters. Eventually lae with as many as 500 cells are reached, but development goes no r. Fig. 212 gives a general survey of the development.

ese results, confirmed and extended on European species of echinoderms[7] n *Chaetopterus* (E. B. Harvey[8]), and by the use of ultra-violet light as a nogenetic agent for the non-nucleate fragments (E. B. Harvey & Hollaender), nstrate that the egg-cytoplasm has within itself the potentialities for at least arly stages of development. Gastrulation and further differentiation, however, seem to require the intervention of the chromatin material.*

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parthenogenetic merogons are devoid of any Feulgen-staining material, but we cannot de from this that the nucleus has not set free any of its contents during the stretching of the nce at this time nearly all the nuclear material of the egg is of the ribo-nucleotide type 633). There must be much of this in the cytoplasm anyway. But it cannot carry the mero- gastrulation, and the external addition of nucleotides, vitamins, and hormones gives it no help.

In connection with this, reference may be made to the question wheth
cleavage rate in echinoderms is a function of the cytoplasm or the nucleu
A. R. Moore[3] reviews the older phases of this, and describes his own experimen
with the cross *Dendraster* ♀ × *Strongylocentrotus* ♂. The hybrid embryos showe
the cleavage rate characteristic of the egg species. By micro-dissection nuclea
and non-nucleate fragments were made, and fertilised either with *Dendraster*
with *Strongylocentrotus* sperm, thus giving homospermic and hybrid diploid ar
andro-merogons. In every case the cleavage rate was that characteristic of th
cytoplasm, even when only a foreign sperm nucleus was present. Substantial
similar conclusions, but less certain because based only on cross-fertilisation
were reached by de Francesco, and for amphibia by J. A. Moore[2].

With regard to the question of the cleavage of non-nucleate merogons
amphibia, Jollos & Péterfi in 1923 removed the egg nucleus by means of
micropipette from fertilised axolotl eggs, and obtained what were apparent
morulae. The sperm nucleus was said to be absorbed. This type of experime
is not represented in Table 24. Doubt was subsequently thrown upon the
results, but Gross recently increased their plausibility by subjecting to co
the eggs of a parthenogenetic race of the brine-shrimp, *Artemia salina*, at th
metaphase state of the first meiotic division. A considerable amount of cleava
resulted, in which the chromatin material did not divide, but remained in or
of the blastomeres only. In some cases "blastulae" were reached.

That there may be substances in the egg-cytoplasm which accelerate, or ar
essential for, cell-division, is suggested by the experiments in which Moore
produced "dumb-bell" eggs (in *Dendraster*) by centrifuging. The cleavage
the centripetal lobe, containing the "oil-cap" and the nucleus, might then eith
be retarded, or the nucleus alone might divide, or neither nucleus nor cytoplas
might divide. Cleavage of the centrifugal lobe went on normally. One cou
therefore assume the existence of a "cleavage substance" sedimenting with th
heavier constituents of the egg (cf. p. 493, on the emission of stimulatory ar
inhibitory substances from echinoderm eggs). Such a mechanism of contr
might explain the hereditary differences in cleavage rate found by Fox[1] betwee
sea-urchin races from different latitudes.

In reading through these sections, the biochemist will have been particular
impressed with the large number of chemical substances (important in many phas
of development), the existence of which has been as good as proved by the metho
of experimental morphology. In the case of the "blood factor" of fertilisatio
a preliminary survey of the properties of the substance has already been mad
In the case of the "histogenetic stimulators", which the haploid embryo lacks an
which contiguity with diploid tissues can provide, the substance or substances a
still to some extent hypothetical. We shall shortly have occasion to describe som
more of these substances, under the head of "nuclear inductors" (section 2·67
But before going on to these other substances, clearly produced by the activit
of the nucleus, it will be convenient to study the question of genic lethality, sin
this has been raised in so acute a form by the experiments already described o
haploid lethality.

2·65. Lethal Genes and organisers

Early in the history of genetics it was noticed that among the offspring of certain crosses, all those which would have been expected to contain a certain segregation of genes appeared to be missing. Investigation showed that they were missing because they had all died before the time of hatching or birth. In this way arose the conception of genes which are lethal to those animals which carry them. Here we shall not find it necessary to go into the genetics of the question; this may be found in the genetical monographs and reviews (e.g. Sinnott & Dunn; Mohr[1]; Landauer[23]; Wipprecht & Horlacher; Morgan, Bridges & Sturtevant). All that I wish to demonstrate is that in many of those cases where the mechanism of genic lethality has been investigated from the embryological point of view, there is ground for the belief that the effect is due to interferences with organiser phenomena.

It is evident that within the limits of what we call the normal, there are many different characters possible for the individual, some relatively insignificant (such as eye-colour), others less so. We know that all these are controlled by genes. At one boundary of the normal there are advantageous variations; at the other there are disadvantageous ones. Disadvantageous variation may be pushed so far as to achieve lethality, so that the organism bearing such genes will be killed, and this may happen at any stage of embryonic or post-natal development. Such genes are called lethal, or if the effect occurs late, sub-lethal. There can thus be no sharp line of demarcation between all these genes, nor can their effects be sharply distinguished from the teratological anomalies accidentally produced in development, except in so far as the regular inheritance of the condition is demonstrated.

Since development is an epigenetic chain of processes, the earlier a gene exerts its effects in development, the more serious its effects will be. And since all animal development is a highly integrated process, it is not difficult to understand why most variations should be disadvantageous, why, in fact, evolution has proceeded "in the teeth of a storm of adverse mutations". The more highly ntegrated a process is, the more difficult it is to alter it without injuring it. At he same time, a very drastic mutation affecting early embryonic stages may occasionally be viable and allow of the occupation of a new oecological niche. Goldschmidt[9] has called such organisms "hopeful monsters", and the possibility of their occurrence in relation to the origin of species was the motive long ago in Bateson's classical collection of morphological abnormalities in his *Materials for he Study of Variation.*

The time at which genes act in the life cycle is indeed variable. Some genes do not act in the life cycle of the organism carrying them, but upon the immediate descendants of the first generation, for instance those genes which affect egg characteristics ("maternal inheritance"). The time at which genes act has been the subject of an ingenious essay by Haldane[1], who suggested the following classification, so far as animals are concerned:

MZ Genes which affect the maternal zygote of the organism carrying them. Influences of the foetus upon the mother would be examples of this; none have so far been studied, but such genetically controlled influences m almost certainly exist.

Z_1 Genes which act early in embryonic development. Often lethal. Examp of these will be given later.

Z_2 Genes which act in larval development. Examples later.

Z_3 Genes which act before the attainment of sexual maturity. The majority genes which have been studied in animals fall into this class.

Z_4 Genes which affect reproductive organs or behaviour, or secondary sex characters. Examples are genes for milk yield or egg yield, and those wh modify the metabolism of the sex-hormones.

Z_5 Genes which affect egg-shell, egg-white or other maternal egg or uteri structures. An example is the egg-shell shape and texture of silkwor (Toyama). "In the evolution of the mammal a critical part was doubtl played by Z_5 genes which interact with *MZ* genes in the foetus to determi a suitable response of the uterus to its contents."

DG Delayed gametic genes, i.e. those which affect gametes, not necessar carrying them, borne by zygotes carrying them. Example: yellowness yolk in silkworm eggs, associated with yellowness of maternal blo (Toyama), and genes affecting egg-shape and fecundity in the fruit- (Crew & Auerbach).

DZ Delayed zygotic genes, i.e. those which affect zygotes, not necessar carrying them, borne by zygotes carrying them. This is true "matern inheritance". Blueness in silkworm embryos is due to pigment formed the serosa, a zygotic structure, but is unaffected by the paternal ger (Toyama). Dextrality and sinistrality of coiling in gastropod eggs such *Limnaea* are *DZ* characters, probably depending on the molecular structu of the unfertilised egg (Boycott, Diver, Garstang & Turner; Diver Anderson-Kottö). In *Drosophila* there is a lethal *DZ* gene affecting fem embryos more than males, so that "whether a given female lives or d depends not on her own genetic constitution but on that of her mothe (H. Redfield). As has been noted already (p. 364) cleavage rate is a proper of the egg-cytoplasm in echinoderms, so that if we may leave cytoplasm inheritance out of account, this also would be controlled by *DZ* genes.

Lethal genes are always harmless in the heterozygous condition, and so a transmitted and perpetuated by organisms which do not suffer from them. the homozygous condition they produce their fatal effects. It does not seem matter how many lethal genes an animal carries in the heterozygous state, as organism will be quite normal though carrying four or more lethal factors, t presence of one alone of which in double dose would kill it (J. Schultz[1] on t fruit-fly; C. V. Green on the mouse).* Lethal genes are important for t embryologist because, as has been well said, they give us "experiments embryology in the reversed direction. While in experimental embryology t experiment is known, but the result is doubtful, here we have to study the resu first and by going backwards to find out what sort of an experiment nature h performed."

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* Some genes *must* be present; an animal some of whose cells lack them entirely ("homozygo for deficiency") will often show local suppressions of these cell groups; these are the "cell lethal of Demerec. Lack of space prevents further consideration of them here, but see below, p. 408.

The following discussion naturally arranges itself in certain sections. We shall liscuss (1) genes causing the death of the embryo before the action of the primary rganiser, (2) genes interfering with the action of the primary organiser, (3) genes nterfering with the action of second- and third-grade organisers, (4) genes roducing anomalies of the individuation field by changes in growth rates of arts, (5) genes producing anomalies of embryonic metabolism, and (6) genes illing or deforming the embryo by unknown mechanisms. The fourth class, enes giving rise to anomalies of the individuation field, may be divided into two. hanges in growth rate may be such as to affect the general plan of the organism nd so have serious morphogenetic consequences, or they may occur quite niformly and smoothly so that they have no such consequences. They may also nvolve the proliferation of one particular tissue at the expense of all the others, nd so give rise to tumours.

2·651. Genes causing the death of the embryo before the action of the primary organiser

It so happens that the classical case of this effect was also the first case of genic ethality to be discovered. Cuénot[2] in 1905 noticed that no homozygous yellow nice were obtainable, and later Castle & Little showed that *yellow* is a gene which nvariably leads to the early death of the embryos if present in the homozygous tate. After a good deal of purely genetical work, it was established by Ibsen & Steigleder, and particularly by Kirkham[1,2], that most of the morulae and blastocysts legenerate and die before implantation has occurred. Such blastocysts have been lescribed by Corner[1]. Should implantation take place the blastocyst is devoured y phagocytes. The causes of these events still remain quite obscure, but as can e seen from modern work on the physiology of the mammalian blastocyst p. 84) many possible mechanisms exist. Another case is that of the lethal nouse studied by Glücksohn-Schoenheimer[2], which survives implantation ccessfully but goes no further owing to a complete failure to form any mesoderm f. p. 359 and p. 629). Since it is associated with *short-tail* strains (p. 374), it ay be regarded as a very extreme form of the mesodermal (notochordal) nomaly exhibited by them.

In insects a large number of lethal genes have been described, but few have een submitted to embryological analysis. In *Drosophila* some of these seem to ill the embryo at an extremely early stage, thus N. P. S. Dobzhansky found that ggs homozygous for *star* develop a black spot in the region of the micropyle and ie before any appreciable development has taken place. Chromosome deficiencies nd aberrations, in particular, such as *nullo-X* and *nullo-IV*, inhibit development t the earliest stages (J. C. Li[1]; Brehme). Similarly in silkworms, some of the ethals described by Nishikawa, by Astaurov, and by Efroimson & Rilova kill the mbryos at the very beginning of development. Poulson[3] alone has investigated he way in which these serious chromosomal abnormalities affect the formation f the germ-layers in the embryo. When the *X*-chromosome is totally absent *nullo-X*), the nuclei fail to divide in the posterior end of the egg, and even in e anterior part they remain densely clumped so that no cell boundaries are ever formed (see the account of insect development later; p. 455). A slightly severe suppression of development is seen when only half the chromosom present (*half-X*); here the nuclei migrate to the egg's surface but no cell-divis appear and no blastoderm. In *notch*-8 the blastoderm forms normally neural tissue hypertrophies, but little endoderm and no mesoderm ever app All such cases will well repay further investigation. It has already been found the respiration of *nullo-X* embryos is markedly reduced (Boell & Poulson).

Serious genic abnormalities, such as the total absence of chromosomes or of chromosomes, absence of genes, unusual translocations and segregations, may also have something to do with the development of tumours (see the cussion below, p. 385, on hereditary tumours, and the previous discussion cancer, pp. 239 ff.). Jones has drawn attention to the probable genetic basis fasciations and some galls (see p. 106) in plants. Conversely, Kostov & Ken have demonstrated irregular chromosome behaviour in galls produced by inse Certain varieties of apples are characterised by well-marked mole-like p jections on the surface of the ripe fruit, due to the egg-laying of the p curculio (*Conotrachellus nenuphar*). The abnormal tissue, an unregulated m of cells, must be different from the fruit tissue, because the scab fungus, instance, will spread up to the margin of the galls but no farther. Some varie of apple respond to the insect's stimulus (are "competent") but others do n and this tendency is transmitted to some of the seedlings.

It has long been known, too, that X-rays, radium, ultra-violet light, heat, other physical agents, produce chromosomal irregularities. But in addition these, non-disjunction of chromosomes has been obtained (e.g. by Mottram) an effect of treatment with the dye gentian violet, with tar, or with the dibenz thracene derivatives, which we have already mentioned as being related both the sterols, and perhaps to the primary evocator (see p. 243). This raises question of whether nuclear anomalies are cause or effect in malignant tissu Boveri[3], in a classical monograph, allotted them an important role, but as may seen from the review of W. H. Lewis[4], the question is still quite undecided.

2·652. Genes interfering with the action of the primary organis

Here the cases known fall into two groups, those affecting the head-organi and those affecting the tail-organiser (see pp. 271 ff.). It must be emphasi that the embryological analyses have in general not been pushed back sufficien far to establish with certainty that this kind of interference has taken place, the disturbances are in some cases so deep-seated that it is difficult to doubt

The most striking case of far-reaching disorganisation of the head-organi is that of the hereditary *otocephaly* in guinea-pigs described by Wright & Ea and later anatomised by Wright & Wagner. Disorganisation is found in ma different degrees (see Figs. 213 and 214). First the ears approach each other a median throat opening is formed. In grade 5 the mouth opening is altoget lost, in grade 6 the nostrils fuse, in grade 8 the eyes fuse (cyclopia), in grade the eyes completely disappear (anopthalmia), and eventually there is no s of a head at all. In all cases the remainder of the body is well formed. T wonderful series of progressive head defects (the genetics of which was later further studied by Wright[4]) must involve head-organiser in the first instance,

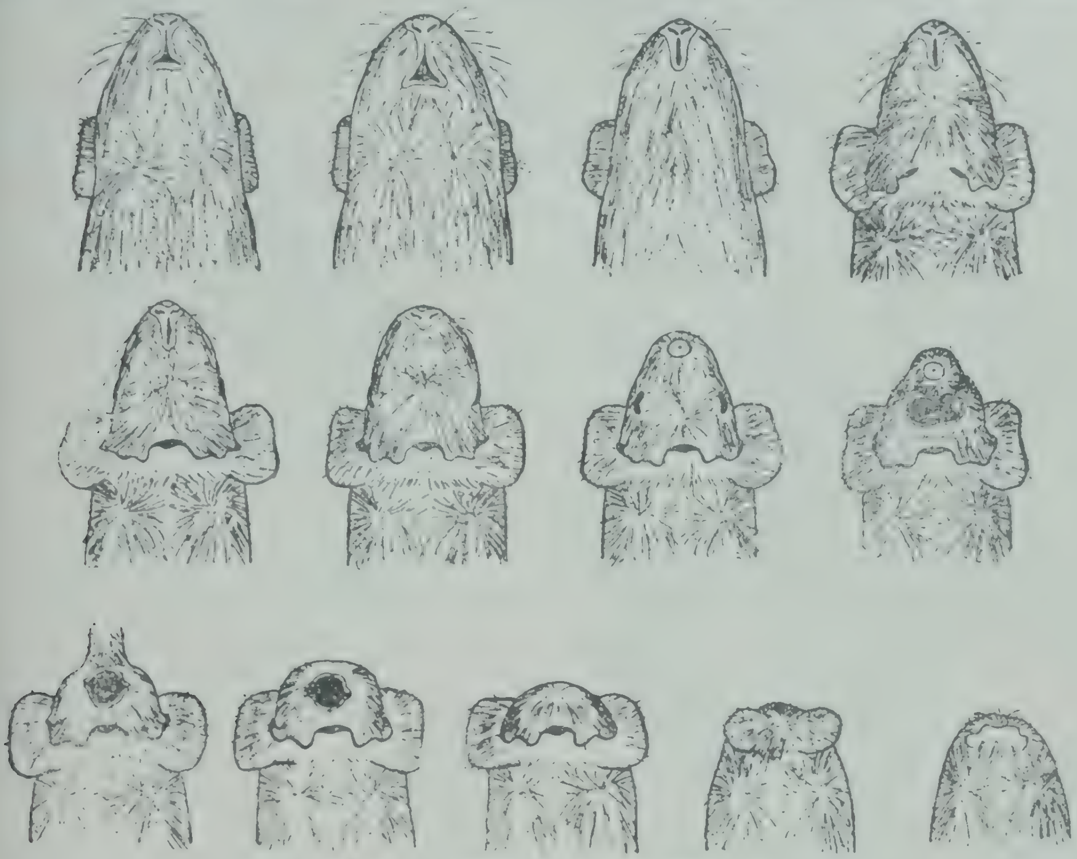

Fig. 213. Otocephaly in guinea-pigs.

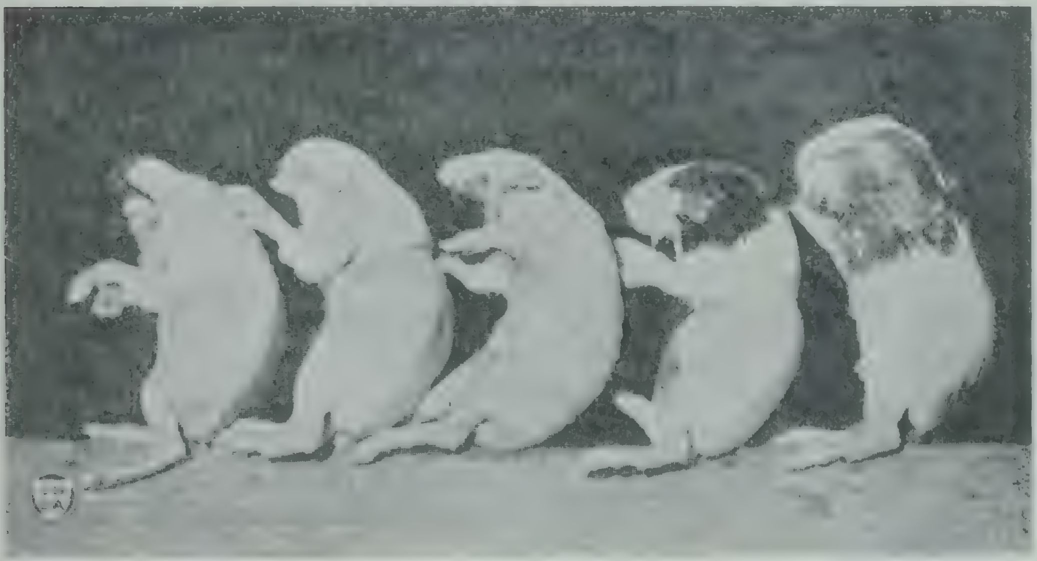

Fig. 214. Photograph of otocephaly in guinea-pigs.

but also the foregut as mouth-organiser, and the mesectodermal cells of the neural crest which normally make the head skeleton.*

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* Perhaps similar genes exist in all vertebrates: cf. Fasten's headless sheep, the *agnathic* lethal absence of jaw in cattle described by Ely, Hull & Morrison, and the human cases of Redenz. Otocephaly can be brought about experimentally by the action of lithium in amphibia; there it is regarded by F. E. Lehmann[7] as due to disturbances of the head-organiser. In rodents direct irradiation of the foetus *in utero* will produce many profound head defects (Job, Leibold & Fitzmaurice).

Wright[5] seems to have felt that the border-line between monsters and gen
abnormalities raised some difficult problems. The American embryologists
and Wilder had considered them in a well-known discussion in 1908. But th
is really no serious difficulty here. A monster, a serious deviation from nor
development, may be caused by some quite external influence, such as an unus
metallic ion in the water surrounding a frog's egg or an unusual endocr
balance in the maternal and foetal blood, or by many relatively mechan
causes. But a gene may be able to bring about just the same deviation, and to
so time after time in a given stock of animals.* In the same way, it has b
found that effects generally produced by genes may be exactly reproduced
acting upon the organism at a certain stage of development with higher or lo
temperatures. Such artificial imitations of gene effects have been called *Phe*
copies (Goldschmidt[8]), because they copy in the phenotype the effects of cert
genotypes. Thus a spontaneous monster would be a phenocopy of the monst
made regularly by a lethal gene (see on, p. 392).

In this connection it is interesting to examine the table drawn up by Hut
Greenwood[2] of the spontaneously occurring monsters in a survey of 12,000 ch
incubations.

	Percentage of total chicks	Percent of tot anomal
Hyperencephaly (absence of eye-cups, cranial roof, etc.)	1·52	41
Exencephaly (failure of skull formation; brain extrudes through the meninges)	0·84	23
Microphthalmia and Anophthalmia (eye-cups abnormally small or entirely absent)	0·81	22
Exencephaly and Microphthalmia	0·25	7
Miscellaneous (including ectopic viscera, malformed limbs, otocephaly, complete absence of head, and various duplicities)	0·25	7

Thus in spontaneous anomalies, failure of the eye-cup inductor seems to be
most common, while the otocephalic abnormalities, up to complete failure
head-organiser and so of all cephalic structures, are relatively rare.

Head abnormalities may also be the result of the segregation of an induc
translocation (Snell[2]; Snell, Bodemann & Hollander; Snell & Picken). Wh
male mice were X-rayed (with a dose of 600 Röntgen units) and then mated
normal females, about a quarter of their progeny were semi-sterile, produci
small litters. These were found to be due to a lethality producing gross m
formations of the anterior part of the neural tube in many of the embryos. Th
is a failure in the closure of the neural folds so that the whole of the fore-bra
mid-brain, and hind-brain may be open (see Fig. 215). The cranial roof is rep
sented only by centres of ossification at the sides of the head, and those pa

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* In an interesting review Wright[5] has elaborated a conception of genetic otocephaly depend
on rates of development (following the thought of Child or perhaps rather Stockard). He dr
a graph showing the time at which developmental inhibitions would have to become effective
produce the various effects observed. This is not inacceptable, but R. G. Harrison, in the d
cussion of his paper, rightly drew attention to the existence of specific inductor substances in
head the action of which the genes must interfere with. Wright & Wagner admitted that
primary factor may be the prechordal plate, the importance of which for head development
been well appreciated as long ago as 1924 by Kingsbury & Adelmann, although at that time lit
or nothing was known of the role of the mesoderm in neural induction.

of the brain that are formed sit later upon the animal's head like a beret (Fig. 216). Such a pseudencephaly appeared about the same time as the American work of Snell and his colleagues in a strain of mice in Norway as a lethal mutation, and was the subject of an embryological analysis by Bonnevie[2]. She succeeded in tracing the anomaly back to the open neural plate stages, in which it appears that the notochord and mesoderm in the head region remain adherent to the neural tissue much longer than in normal embryos. If the lethals do not die at this stage, pseudencephaly results. The neural tissue seems to be unduly abundant

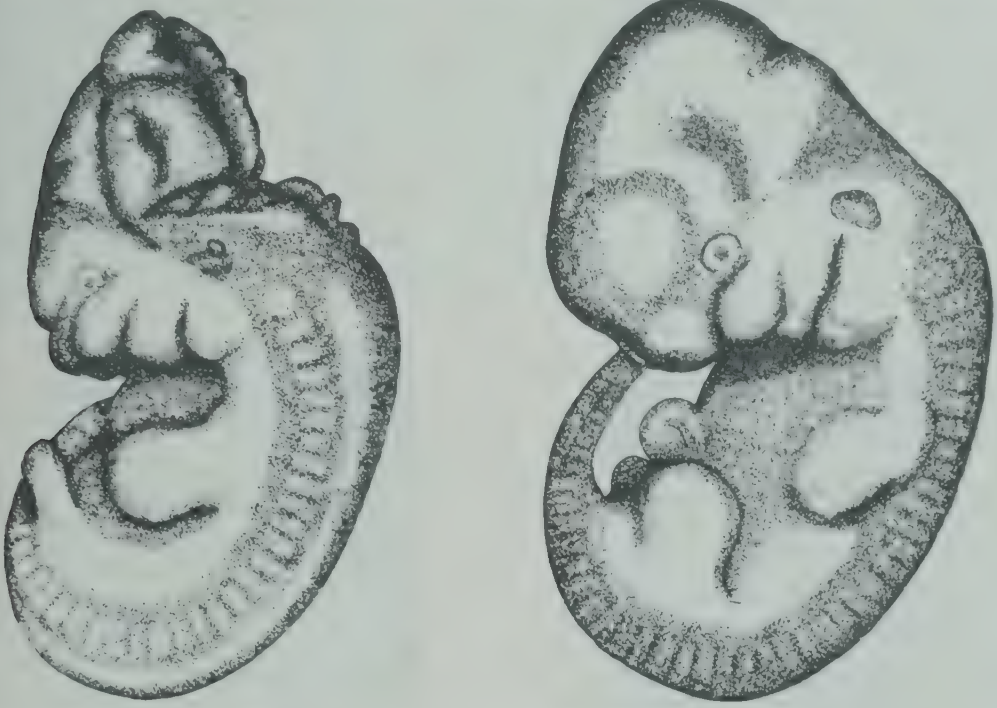
Lethal 12¼ days Normal 12¼ days

Fig. 215. Failure of mesencephalon and rhombencephalon to close in progeny of X-rayed mice.

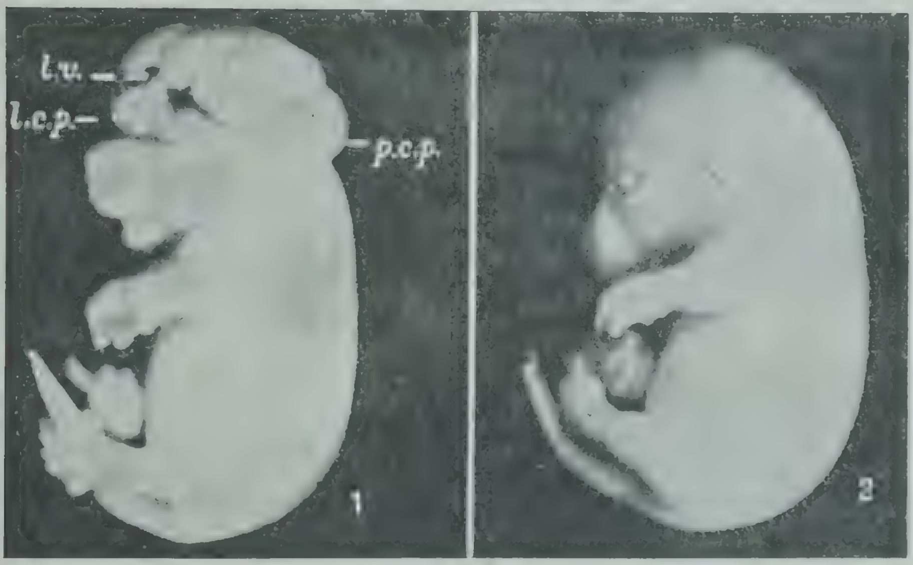

Fig. 216. Pseudencephaly in mice (14½ days). 1. Abnormal strain. 2. Normal. *l.v.*, lateral ventricle; *l.c.p.*, lateral choroid plexus; *p.c.p.*, posterior choroid plexus.

in relation to the head mesoderm. Even so, the exact nature of the failure of t brain folds to close remains obscure,* and an understanding of it will probab have to await a better knowledge than we have at present about the process going on in the normal closure of the neural folds (see p. 334). At the same tir it is interesting to compare this genetically caused failure with the results Holtfreter[10] on *Nachbarschaft* already referred to (p. 284), and the failure closure seen sometimes in embryological experiments. It is almost sure that v have to deal here with some anomaly of the head-organiser. Lastly, it m be added that failure of the neural folds to close has been described in a spo taneous human case (1-month embryo) by Orts. There is indeed a possibili that pseudencephaly in man may be hereditary.

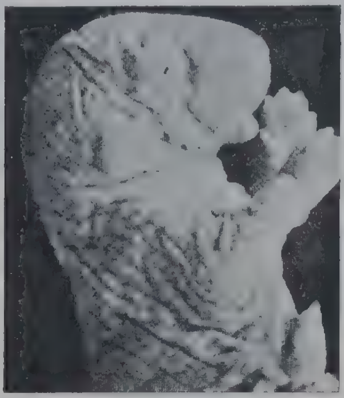

Fig. 217. Lower jaw reduction in progeny of X-rayed mice.

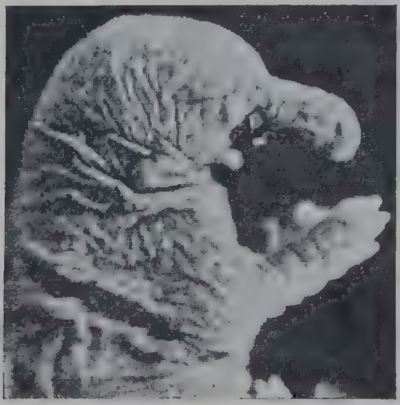

Fig. 218. Head anomalies in progeny of X-rayed mice.

Now it is interesting that an extremely minor degree of the same phenomeno (lack of complete closure of the neural canal) has been shown to be largel responsible for one of the best-known genetic lethal multiple anomalies, the Bagg Little foot and eye "syndrome" in mice, which itself involves far-reaching ar serious defects. In 1924 Little & Bagg obtained a strain of mice after X-ra irradiation which showed the presence of lethal genes causing the followin effects: (*a*) anomalies of the eye and face, (*b*) anomalies of the limbs, includir club-foot and digital reduplications, (*c*) anomalies of the hair in the saddle regior and (*d*) disorganisations of the head, including severe reductions of the jaw. Thes latter gave some of the embryos a peculiar "elephant-trunk"-like appearanc (see Figs. 217, 218). Some years later Bagg[2] showed that the limb anomalies wer associated with the presence of blisters and blood clots at the extremities, whic interfered with the fore- and hind-limb "fields".

At this point the embryology of the condition was taken up by Bonnevie[1]. Sh showed that although the development of the nervous system proceeds almos perfectly, there is a remarkable expulsion of cerebro-spinal fluid from a forame in the roof of the hind-brain. Such a foramen is normal in some mammals, bu in the lethal mice, it allows a large quantity of the fluid to escape, forming bliste under the epidermis, first on the dorsal surface of the head and then graduall

* Experimental transplantations are really needed to elucidate the anomaly in its earliest stage but of course these would be very difficult to do on mammalian material.

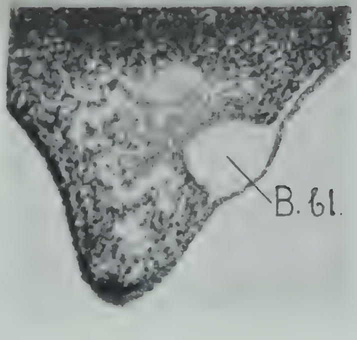

Fig. 219. Sub-epidermal blister formed by escaped cerebro-spinal fluid in progeny of X-rayed mice.

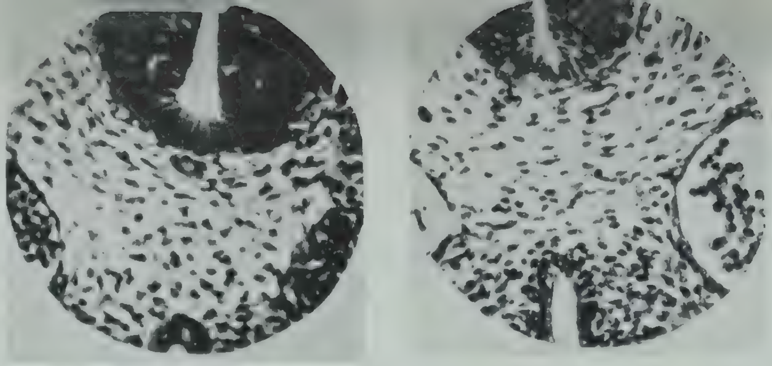

Fig. 222. Suppression of notochord in *short-tail* mouse embryo.

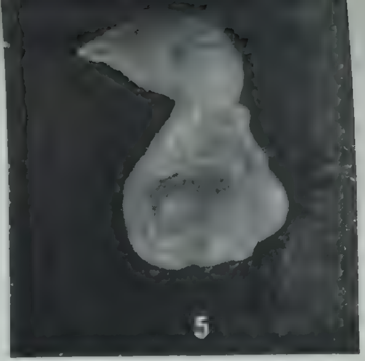

Fig. 223. Suppression of limb-buds in *creeper* chick embryos.

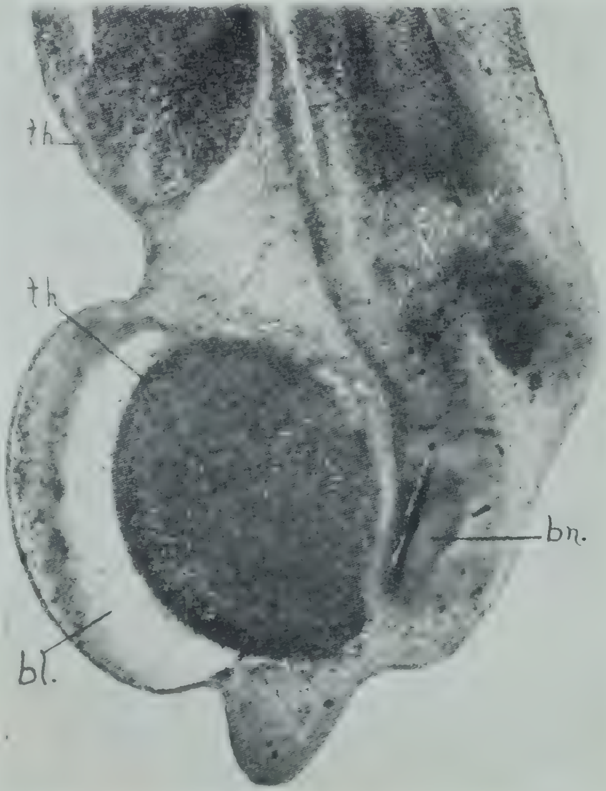

Fig. 220. Haematoma and sub-epidermal blister.

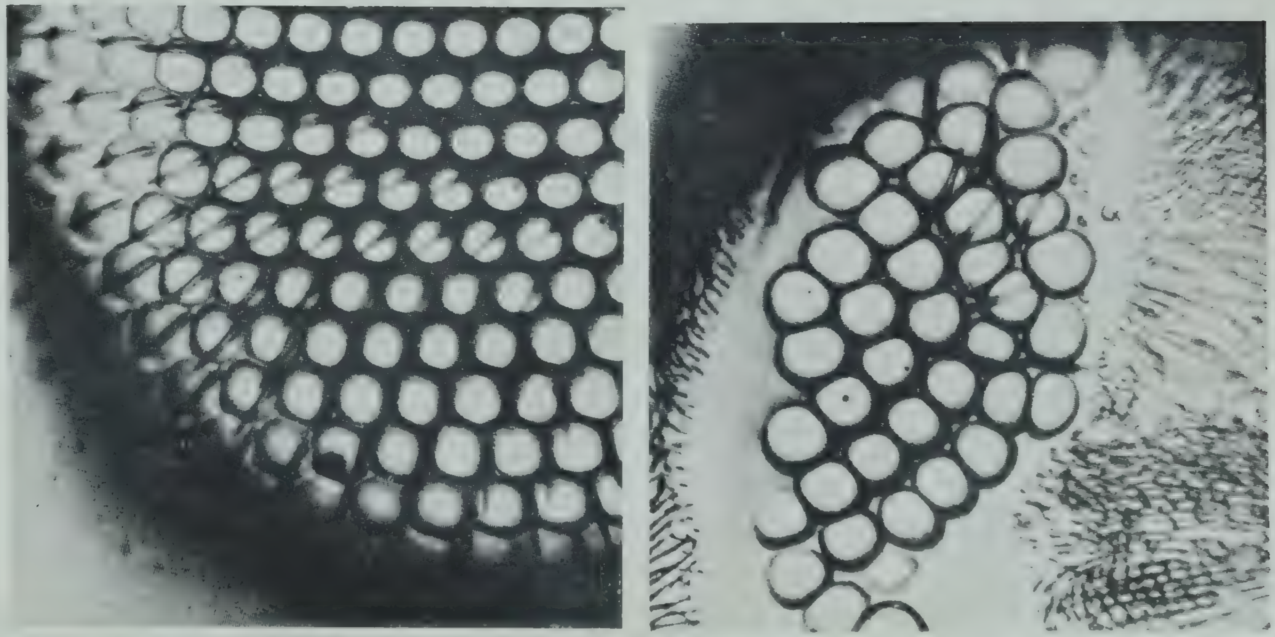

Fig. 233. *Bar* allelomorphs in the facetted eye of *Drosophila*.

spreading all over the surface of the body. Some of the blisters move into the limb-bud districts and so are carried into the limbs, where they give rise to syndactyly, polydactyly, etc. and interfere in various ways with the normal limb pattern (see Fig. 219). Others move in front of the eyes and so prevent the induction of the cornea by the eye-cup and lens. As time goes on the capillaries on the floor of a blister weaken, discharging their blood into it, and forming in many cases haematomata. Fig. 220, taken from Plagens, who attached much importance to the bleeding, shows such a haematoma pressing into a fixated blister. The genetic control of these effects is complicated, for it seems to be possible to breed a strain in which eye defects predominate over limb-bud defects, and another in which the reverse is true (Little[2]; Little & McPheters). Moreover, in the strain of Bagg & Little, the eye defects were much more severe than in the material studied by Bonnevie, including even the absence or atrophy of both eye-cups, which could not possibly be referred back to the effects of blisters. Similarly, in one of Bagg's strains (Bagg[1]), the foot and eye anomalies were accompanied by the complete absence of one or both kidneys, and this could be intensified by selection.

In this interesting case, therefore, we have to deal with a number of effects, some of which can be brought together as the results of one deviation from normal development, namely, the expulsion of cerebro-spinal fluid from a weak spot on the roof of the hind-brain. For this reason we have classified the Bagg-Little strains under the heading of primary organiser anomalies, but it is clear that second-grade organisers seem to be involved too, particularly the eye-cup organiser, the pronephros-metanephros organiser system, and the inductor of the cornea.

It may be noted that cerebral hernia, a mild degree of pseudencephaly, has been found to exist as an inherited sub-lethal factor in pigs (Nordby) and cattle (Shaw). What is to some extent the converse, hereditary hydrocephaly, is inheritable in mice (F. H. Clark; Zimmermann).

Another case which gives us a transition to interferences with second-grade organisers is that of the *shaker-short* mice. "Waltzing" mice occur in various well-known genetical types, such as "circlers" and "shakers", each type having a characteristic movement disturbance and a characteristic deafness. The "short-tailed shaker" type arose as a mutation and according to Dunn[4] is due to one recessive gene. Its embryological analysis, undertaken by Bonnevie[3], showed that, owing to a weakness of the roof of the brain (cf. the pseudencephaly and Bagg-Little types just described), marked hernia containing cerebro-spinal fluid and blood occurs. Hence the normal pressure of cerebro-spinal fluid within the brain cavities does not arise, and there is no outward movement of the brain walls, as in normal embryos (see Fig. 221). Hence the ear-vesicles, instead of being compressed to a more discoidal shape, tend to remain spherical, as they were when they were first induced. From the experimental work of Kaan we know that although the ear-vesicle may after its first induction take the initial steps in the formation of the labyrinth, the formation of a normal organ depends on its relations to neighbouring structures. And so all the later distortions of the internal ear in *shaker-short* mice were found by Bonnevie to follow from th primary failure of expansion on the part of the brain. Thus they form no end lymphatic appendage, which is probably normally induced by the hind-brain.

We come now to the genetic disturbances associated with the caudal end the body; interferences with tail-organiser. The well-known *short-tail* gene i mice is lethal when in the homozygous condition. An account of its first discove and a review of its genetics has been provided by Zavadskaia, Koboziev Veretennikov. What we know of its embryology is mainly due to P. Chesley, wh found that homozygous *short-tail* embryos die at 11 days gestation and a resorbed. The first signs of anomaly appear at 8½ days as small blisters on eac side of the mid-line, which later disappear. There then arise marked irregulariti of the neural tube, which sends out branches some containing a lumen, som not; the somites are not well formed, and posteriorly completely absent; the are no hind-limb buds. In section, it can be seen that the differentiation of th

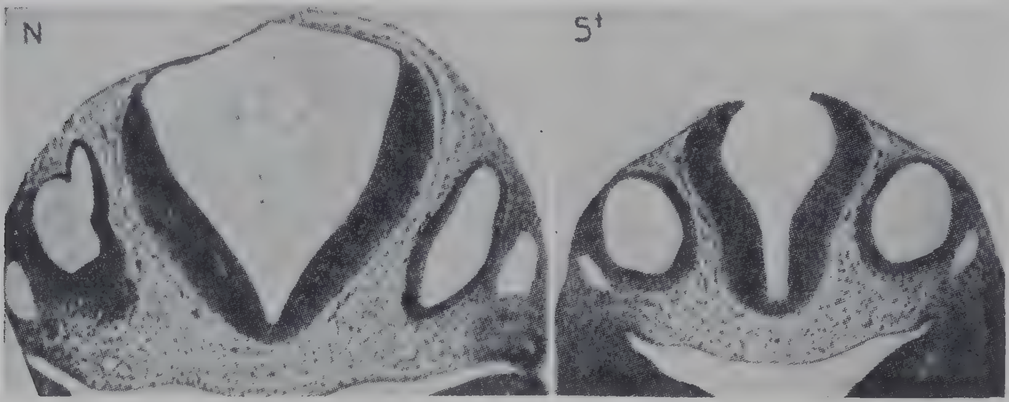

Fig. 221. Cross-sections through the head of a normal mouse embryo and a *shaker-short* one.

notochord is very deficient (see Fig. 222, opp. p. 372); the picture is thus rath reminiscent of the notochords suppressed by lithium in Lehmann's work[6] (p. 226 Here, however, disorganised neurulation follows. We are bound to recognise a interference with the primary organiser.

Heterozygous *short-tail* embryos have been investigated by Glücksohn-Schoenheimer[1]. Chesley had shown that their tails atrophy as the result of a sharpl marked constriction, and Chesley & Dunn had studied their genetics. Glücksohn Schoenheimer found that this constriction appears at the 11th day, but is histo logically visible one day earlier, for sagittal sections of the tail bud reveal th although the neural tube has grown back some way into the tail, the notocho has not. Here again an anomaly of the notochord, the primary inductor, lea to the failure of an axial structure.

Numerous lesser degrees of inherited tail abnormalities have been describe The tail of the *flexed-tail* mouse (Hunt & Permar; Hunt, Mixter & Permar) fixed in one or more right-angle bends due to the unilateral fusion of adjace vertebrae (Kamenov), owing to the presumptive inter-vertebral discs becomin bone instead of fibre. Kamenov, however, could not show that the anomaly w caused by any anomaly of the notochord. *Flexed-tail* involves, for reasons at present unknown, considerable anaemia (Mixter & Hunt), due both to haemoglobin deficiency and lack of erythrocytes. A gene with similar effects, *wry-tail*, exists in cattle (Atkeson & Warren).

Trunk-organiser may be affected as well as the primary organisers of head and tail, if we may interpret in this way the *short-spine* anomaly in cattle described by Hutt[2].

The other well-known case which might be classified as tail-organiser failure is the gene *rumpless* in fowls, the genetics of which have been studied by Landauer[12] and by Dunn & Landauer[2]. Known since the seventeenth century, this condition involves the absence of all the synsacro-caudal vertebrae posterior to the third, and the complete suppression of the five free caudal vertebrae, together with the uropygial (oil) gland and other neighbouring structures. From the work of du Toit we know that the segments missing from the adult fowl are missing from the tail bud of the 3rd day embryo. Intermediate grades, also hereditary, between complete rumplessness and the normal, exist (Landauer[2]). It is interesting that according to Dunn[2] rumplessness occurs spontaneously from time to time in large chick populations, and Danforth[2] has succeeded in producing phenocopies of *rumpless* by subjecting developing eggs of normal chicks to abnormally high temperatures during the early incubation stages.

2·653. Genes interfering with the action of second-grade organisers

In the course of the preceding discussion we have already seen a number of cases of this, mostly concerning the organs of the head. The extreme cases of otocephaly of Wright & Eaton must involve failure of eye, ear, and mouth inductors. The eye-cup suppressions in some of the Bagg-Little strains may be due to failure of the eye-cup inductor, and the kidney suppressions may, indeed must, be interpreted in a similar way. Certain hybrid guinea-pigs also may show complete anophthalmia (Eaton; Pictet & Ferrero) and mice too (Chase & Chase). Genetic eye-cup suppression may be compared with that already noted as occurring in vitamin-B deficiency in pigs (p. 227); possibly the gene and the vitamin act upon the same mechanism in different ways.

Other inherited defects in head organs are the useless chick mandibles described by Asmundson[2] (this is a lethal gene, since feeding is not possible), and the deficient teeth in the *grey-lethal* mice of Grüneberg[1], which will be referred to again. Congenital absence of all teeth in man is known (Thadani). The failure of cornea induction in the Bagg-Little strains as analysed by Bonnevie[1] was due to mechanical separation of inductor and competent tissue by the travelling blister,* but cases of more subtle interference with cornea induction are known, such as that reported by Whitlock. In a certain guinea-pig strain, the presumptive cornea, though normally underlain by lens and eye-cup, never achieves the transparency typical of the normal cornea, and differentiates into epidermis instead. A particularly interesting genetic anomaly, which involves the persiste of the gill-slits in sheep, giving fistulae instead of wattles, is described Vassin.

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* Cf. the identical separation of inductor and reactor in the blindness of cave forms, already referred to (p. 225).

In insects also the development of a head organ, such as the eye, may fail, in *Drosophila* (Derrick[1]) and *Habrobracon* (Speicher). An *eyeless* fruit-fly has t two inner optic ganglia on each side but the outer one has disappeared; the may be no ommatidia and no facets (Richards & Furrow). Derrick showed th the eye-rudiment is greatly reduced in size from its first appearance. Moreov the moment of first appearance is later than normal.

But the most many-sided genetic deficiencies in a second-grade district a those of the limb-buds. They may be entirely suppressed, as sometimes occu in *creeper* chick embryos (Landauer[17])—see Fig. 223, opp. p. 372—to whi we shall return, or in the cat (Kirkham & Haggard) or man (Ilberg). Or dis parts may be suppressed to varying extents, as in the absence of hands or feet (acheiropodia) or of the digits (brachydactyly), and the digits may fail to separate (syndactyly). On the other hand, reduplications may occur, as in polydactyly.

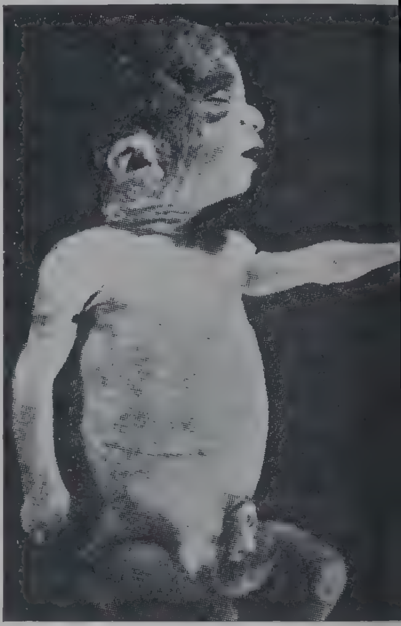

Fig. 224. Suppression of limbs human development.

Suppression of distal parts of limbs in mammals used to be interpreted as being due to "intra-uterine amputation", i.e. strangulation of the limb by amniotic folds, umbilical cord, etc. But Streeter has conclusively shown that such "amputation" is really a failure of formation, due to defective limb-bud histogenesis followed by de-differentiation and healing of the stump. In man (where the end-result may be as pictured in Fig. 224) defects tend to be present in all four extremities if in one, and there is some likelihood that the condition may be hereditary. In the rabbit genetic brachydactyly and acheiropodia has been studied by Greene & Saxton. Nothing is visibly abnormal before the 18th day of gestation, but then there occurs vascular breal down and extravasation of blood followed by necrosis. In this case, therefor a limb-bud organiser cannot be incriminated, and we are reminded rather the vascular degeneration which follows vitamin-E deficiency (see p. 265) embryos.* Genetic failure of the limb-buds to form beyond the first joint has bee described in cattle by Wriedt & Mohr. This was associated with extraordina deformations of the face, giving the calves the appearance of parrots.

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* The appearance of limb buds followed by their complete or almost complete resorption m occur not as the result of a distinct lethal gene, but in crosses. Thus Hamburger[1] has report such phenomena in newt hybrids (*taeniatus* ♀ × *cristatus* ♂ and *palmatus* ♀ × *cristatus* ♂), a Montalenti in toad crosses. Suppression of limb buds, as it imitates what may happen genetical may be looked on as a "phenocopy" phenomenon (see p. 392). It has been brought about selenium injections in the case of the chick (Franke, Moxon, Poley & Tully) and by 10 % sucro in the case of amphibia (Sladden).

That lethal genes may involve organiser excess as well as organiser suppression appears clearly from the work of Wright[2] on polydactylous guinea-pigs. Guinea-pigs usually have four toes on the front foot and three on the hind foot. By selection within a race containing a gene producing supernumerary fingers and toes, Wright obtained a strain in which the homozygotes invariably died during the foetal period, after producing paddle-shaped feet bearing from nine to twelve digits each, while the heterozygotes showed the pentadactyl foot. In the homozygous condition, the embryos also had head defects including micropthalmia. These interesting embryos were studied by J. P. Scott, who was only able to find, however, that the supernumerary centres of digit-formation appeared in the earliest stage possible. He interpreted the effects as due to an arrest of morphogenesis and alteration of relative growth rates, but the evidence for this is not entirely convincing and growth rates alone will hardly account for reduplications of morphological entities. It must be noted that the polydactyly is not the cause of death, which occurs about the 26th day of development. The cause of death is the bursting of blood-vessels in the region of the shoulder-hump, a mass of connective tissue behind the brain which is the site of a large fat body in the normal newborn animal. The haemorrhage quickly spreads to other parts of the body. Genetic polydactyly in mice is described by Danforth[1] and by Droogleever-Fortuyn; it apparently involves slight corresponding abnormalities of the central nervous system (Y. C. Tsang), and is not due locally to travelling blisters but rather to abnormally numerous growth centres (T. K. Chang).

Polydactyly occurs rarely in man, where it may possibly be hereditary (cf. the discussion in Scott's papers). Club-fingers in man are certainly hereditary (Witherspoon[2]). Club-fingers produced by disease might be regarded as phenocopies of this.

In insects genes causing distortions of the appendages are well known. Thus Hoge discovered a gene producing reduplications of the legs on each segment in *Drosophila*, and the gene *crippled*, described by Komai for the same animal, auses all kinds of leg abnormalities: twisting, shortening, forking, etc. and in ome cases complete suppression (cf. "amputation"). But the most remarkable henomena of aberrant leg effects in insects are perhaps those of "hetero-morphosis", especially *aristapedia*. Regenerative hetero-morphosis has long een known. After extirpation of the eye, an antenna may be regenerated instead f the missing eye (Herbst[3] on *Palaemon*; Křížinecký on *Tenebrio*), and the new organ may be functional (Lissmann & Wolsky). After removal of an antenna a arsus may be regenerated instead (Przibram[2] on *Sphodromantis*; Cuénot[3]; Brecher; nd Borchardt on *Dixippus*). But Balkaschina discovered a recessive gene in *Drosophila* which regularly produces a leg or tarsus from the presumptive antenna rudiment. Normally segmentation of the antenna in its imaginal disc begins on the fourth day of larval life while that of the tarsus begins on the second day. In the *aristapedia* flies, however, segmentation of the antenna begins two days earlier than it should. This time-difference might account for the change, as uggested by Goldschmidt[8] and Braun, by giving one inductor opportunity to ct out of its due order.

One of the most interesting features of *aristapedia* is the fact established Waddington[25] that genes which normally act upon legs act just as well upon t *aristapedia* leg. Conversely, an animal may be produced in which only t proximal part of the antennal appendage is a leg and the distal part is an antenn it is then found that genes such as *thread*, acting on antennae, exert their effe perfectly in this very circumscribed and isolated locality.*

An analogous anomaly is that of *proboscipedia*, where the mouth organs alwa assume the character of tarsi (Bridges & Dobzhansky).

The experimental embryology of amphibia has shown that we may speak a blood inductor (see pp. 164, 301 ff.). Perhaps such a second-grade induct is affected in the lethal gene *anaemic* of mice first discovered by Little[1]. That kills embryos bearing it at birth or in the first post-natal week was established de Aberle and Detlefsen. Analysis of the effect by Gowen & Gay[†] showed th although the haemoglobin content of the erythrocytes is normal, only one-thi of the normal number of these is present (see pp. 648 ff.). This certainly points t deficiency in the inductor-reactor system of the blood cells. The lethal embr are not deficient in iron, and feeding iron or liver to the mothers has no benefic effect. Repeated injection of blood into the peritoneal cavity of the new-bo embryos was found, however, to prolong life almost indefinitely (cf. the administr tion of pituitary to genetic dwarfs, p. 390).

Yet another condition indicating an induction failure is that of "nakedness in organisms normally covered with a hairy or furry coat or feathers. Genes f this, usually sub-lethal, are particularly common; they have been described, f example, in the rabbit (W. E. Castle), the pig (Roberts & Carroll), the mouse (Cre & Mirskaia), the rat (Roberts[2]), the cow (Mohr & Wriedt), and the chick (Hutt Hutt & Sturkie). In the last-named case, the gene for *naked* completely inhibi the eruption of the feathers from their follicles, and is 50 % lethal during t last 2 days of incubation. Feeding cysteine and cystine to the laying hen h no effect. Skin from naked chicks transplanted to normals was found to determined—no feathers could be produced from it; and *vice versa*. In t *hairless* rat, the condition is associated with disturbances of the oestrous cycle, a the organ weights differ considerably from normal animals (F. E. Emery), proba because, as Benedict & Fox have shown is the case in the *hairless* mouse, the ba metabolism is very considerably raised above normal. To the histology of the genic effects an elaborate study was devoted by L. T. David, who found th there could be either imperfect keratinisation breaking off the hairs as th emerged from the follicles; or degeneration of the follicles; or a failure of t formation of the follicles.

More serious skin anomalies than nakedness are also known. For examp in cattle, the gene or gene-complex *epithelial* causes defects in the skin and muco membranes at sites which soon become infected after birth (Hadley & Warwic In man the outstanding example is the condition known as *ichthyosis congenit* where a colossal hyperkeratosis transforms the skin of the new-born child in a leathery coat, traversed by bleeding fissures (see Fig. 225 from Lesser). The literature on this and similar anomalies can be traced through the review of Mohr[1].

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* Phenocopies of aristapedia have been produced by Rapoport[2] using boron compounds.

† See also Smith & Bogart for biochemical data.

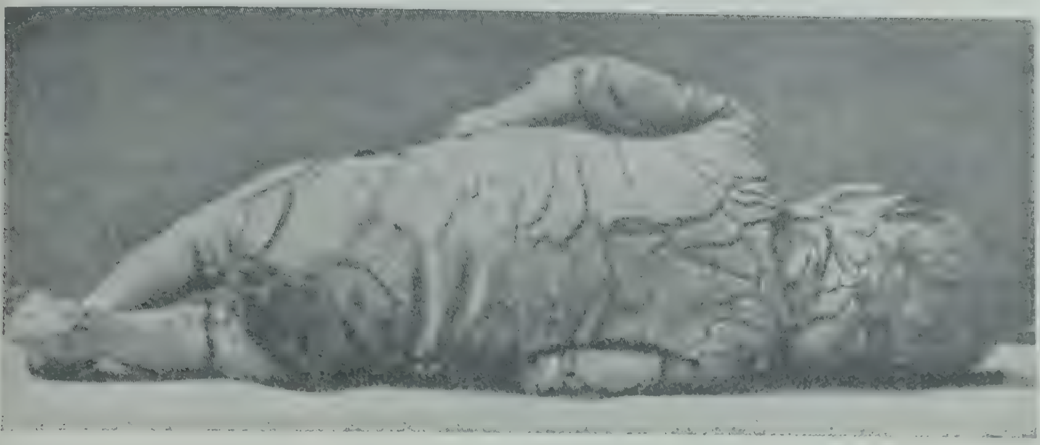

Fig. 225. The skin anomaly *ichthyosis congenita* in man.

2·654. Genes producing anomalies of the individuation field by changes in the growth rates of parts

In the first place it must be realised that changes in growth rate during development may be genetically brought about in such a way as to cause no morphological effects. Pure magnification or reduction of the pattern of the organism need involve no distortion. Thus in *Drosophila*, *giant*[*] (Gabritschevsky & Bridges), where the larvae continue to feed and grow longer than normal larvae, may be regarded as the opposite of *vestigial* (Alpatov[1]; Dunn & Coyne), where larval growth is stunted. In neither case is morphogenesis interfered with. The same applies to *dwarf* in mice (see p. 390).

Alternatively, the shape of the organism may be changed by a change in shape f some part, such as the muscle masses, without appreciable disorganisation of orphogenesis. Such seems to be the case in *Drosophila* with the gene *chubby* T. Dobzhansky & Duncan), where the shape of the eggs is the same, the growth ate of the embryo is the same, but the shape at birth is different (shorter and roader), because the muscles are laid down in shorter and broader form. nother gene, *rudimentary*-12 (N. P. S. Dobzhansky), simply shortens the wings, ut has no effect on general growth rate.[†]

But if changes in growth rate affect directly one developing part and not the thers, or if a general change in growth rate occurs at a time when some processes re proceeding much more rapidly than others, so that the former are preferentially affected, then serious disturbances of morphogenesis will ensue. Of this character appears to be the lethal homozygous *creeper* gene in fowls, perhaps the most thoroughly analysed of any lethal hitherto discovered, owing to the work of Landauer, Dunn, and their collaborators.

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[*] But cf. *lethal-giant* already mentioned (p. 361).

[†] The most outstanding cases of genetic shape distortion without morphogenetic disturbance are ound in plants, as Sinnott's studies[1] of fruit shape show.

Chondrodystrophy may take milder forms, of which the nutritional micr melia described by Byerly, Titus, Ellis & Landauer, and anatomised by Landauer[1] for instance, may be an example.

With these nutritional malformations as a background, we may now consid the genetically produced extreme form of chondrodystrophy known as hom zygous *creeper*. The Creeper (Scots Dumpie, Courtes Pattes, or Krüperhuhn) a long-known breed of fowl characterised by a striking shortness of its extremitie The shaft of the tibia is bent, and a complete fibula (normally rudimentary) present; the toes are curled. In the shafts of the long bones there is no end chondral ossification. That the *creeper* gene was lethal in the homozygous sta was first suggested by I. E. Cutler, and definitely established in later breedin experiments (Dunn & Landauer[1]; Landauer[5,10]; Landauer & Dunn[2]). The ca is thus analogous to the *yellow* mouse, which never breeds true.

Landauer made a detailed anatomical and histological study of the developme of the skeletal system in both heterozygous and homozygous *creeper* chic (Landauer[6,11]). From the first of these it was suspected that growth ra changes played a very significant part, particularly since the greater was th normal length of a long bone, the greater was its relative reduction in the Creepe Exact measurements of this kind were carried out by Landauer[14] who foun that the long bones of *creeper* chicks were always shorter than normals, and th the more distal they were, i.e. the later they arose in development, the mo serious was their relative reduction. Lerner's heterauxetic calculations[1] confirme this conclusion. Study of the homozygous lethal revealed more far-reachin anomalies; great reduction of wings and limbs, reaching almost to their su pression; fusion of radius-ulna and tibia-fibula; failure of formation of epiphysi cartilages; no trace of ossification. Eye abnormalities accompanied the skelet ones, especially coloboma, to which Landauer devoted a special paper[8].

When Landauer[7] examined the earliest stages of the homozygous *creeper* embry he found that it was always demonstrably smaller than normal, even as early 36 hr. incubation (cf. Fig. 227). He concluded that the primary action of th *creeper* gene was to produce a general inhibition of growth, and that the lim buds, which were growing most rapidly at the time of inhibition, consequent suffered most severely. This view was tested by Fell & Landauer, who cultivate limb-buds and mandible rudiments from 3–5-day normal chick embryos in growth-restricting medium (plasma-saline with very little added embryo extract Such limb-buds showed after a fortnight's cultivation many of the abnormaliti characteristic of *creeper*, e.g. tibia-fibula fusion, retardation of cartilage differenti tion, absence of perichondral ossification.*

An interesting attempt to get further light on the chemical mechanism involved in *creeper* deficiencies was made by Landauer, Upham, Rubin Robison, who estimated the bone phosphatase in normal and *creeper* chick But there was no difference between the two. Nor did the injection of aqueo

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* Mandible rudiments, however, developed normally, just as they do in *creeper* homozygote thus showing that conditions which prevent normal ossification of cartilage do not preve ossification of membrane bone.

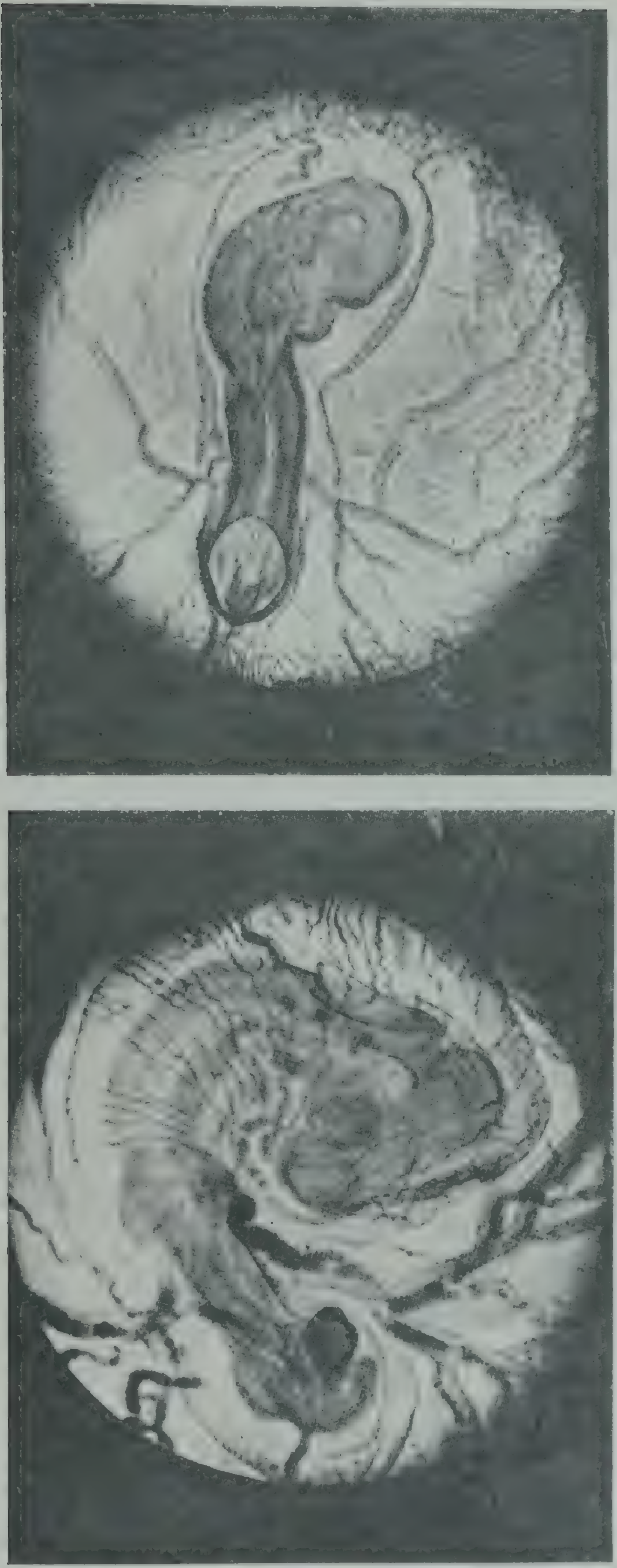

Fig. 227. Retardation of growth in *creeper* chick embryos.

bone extract to heterozygous *creeper* chicks have any effect on the growth of th limb bones. Sex hormones are perhaps unlikely to be involved in the syndron as castration has no effect on either normal or *creeper* bone growth (Landauer

The relation between vitamin-D deficiency and *creeper* chondrodystrop has also been studied by Landauer[15], who found that *creeper* chicks sh symptoms of rickets some time before normal ones, and that their rickets is m pronounced. The chondrodystrophic condition prevents the ameliorati responses to D-deficiency which occur in normal embryos. Similarly, Landau showed that irradiating eggs with ultra-violet light had no effect on hon zygous *creeper* embryos, but improved the hatch of the heterozygous ones to same extent as that of normal embryos.

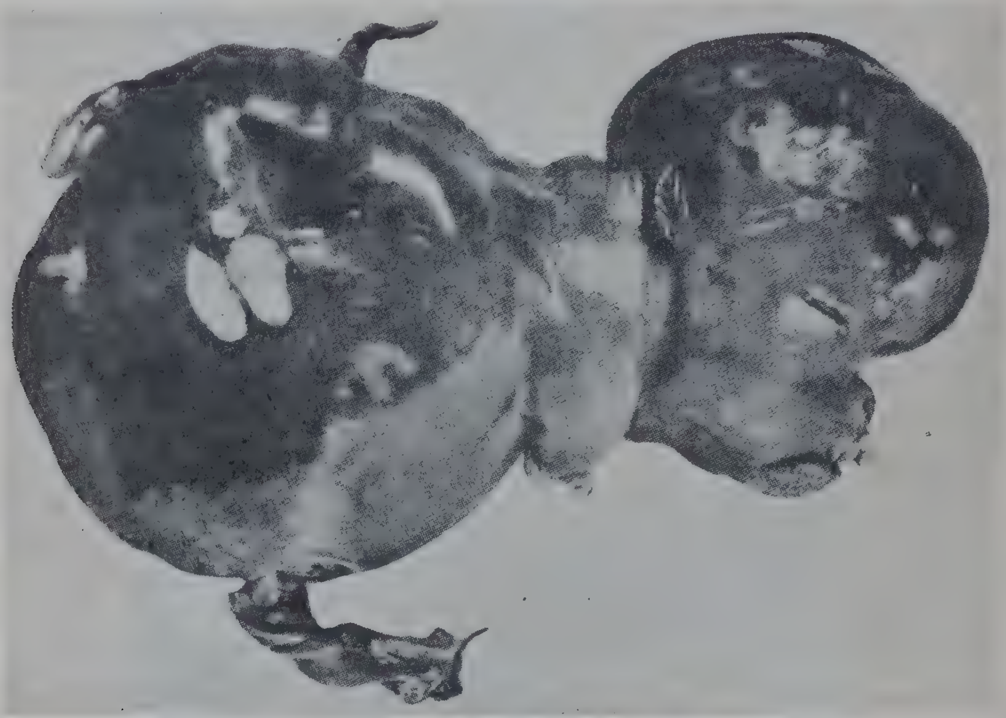

Fig. 228. Achondroplasia in the "bull-dog" calf.

The homozygous *creeper* condition is, as has already been said, lethal. It the fore invited explantation or transplantation experiments, designed, like those Hadorn and Ephrussi already described (p. 359), to test what powers of norn differentiation existed in the homozygote unable to manifest themselves account of its lethality. This problem was studied by P. R. David, who fou that isolated fragments of the homozygote embryo, representing practically ev tissue of the body, would, when cultivated *in vitro* or as chorio-allantoic gra survive long beyond the ordinary lethal period of their donor. Heart fragme showed an unusually low growth rate, but the other tissues behaved normally this respect. David concluded that the death of the homozygotes was due, to cellular inviability, but to some dislocation of normal correlations caused the growth-rate changes and the differential reactions to them.

Analogous experiments, due to Hamburger[2], consisted in transplanting hon zygous *creeper* limb buds at 2½ days' development to normal embryos. Surviv long after the presumptive death of the donor, was observed, but marked *creeper* effects, such as phocomelia, occurred. In control transplants (normal to normal) growth inhibition took place but no *creeper* effects. All these experiments show that the cause of lethality resides outside the limb area. Whether a general inhibition of growth will really account for the effects is still doubtful.

It seems that there is a general similarity between the Creeper breed, Dark Cornish (Landauer[18]) and Japanese Bantam (Landauer[24]) in homozygotic lethality.

In mammals achondroplasia is of course well known, and the great monograph of Rischbieth & Barrington proved that in man it is inherited. Achondroplasic dwarfs have a complete absence of the cartilage columns in growth zones of the long bones, and a mucoid degeneration of the matrix. In cattle, the Irish Dexter breed has long been known to produce achondroplasic foetuses, which are called "bull-dog" calves. Fig. 228, taken from Crew's anatomical study of them[1], shows the strange appearance which they possess, with the feet close to the body owing to the absence of limb elongation. The heterozygous Dexter animals show the same blood picture as that of chondrodystrophic chicks (Landauer & Thigpen). Bull-dog calves occur in other breeds besides the Dexter (Carmichael) and Lipsett reports achondroplasia in urodeles.

2·6545. Hereditary tumours

The *creeper* condition is demonstrably one in which a general growth retardation bears particularly heavily upon those organs which are growing most rapidly at the time when it occurs, namely, the limb-buds. When we turn to the second class of individuation field anomaly, the hereditary tumour, we find an opposite state of affairs, namely a specific growth acceleration of certain small groups of cells.

It is in *Drosophila* that the classical hereditary tumours occur. In 1916 Bridges[1] noticed that a certain sex-linked gene, *lethal*-7, caused the appearance of intense black spots within the larvae, which died in great numbers. Closer analysis of he condition was undertaken by Stark[1], who found that the black spots were rowths resembling melanoma. They originate mainly in the imaginal discs Fig. 229 shows a tumour developing in the two anterior discs), but also in the proventricular ganglion or the salivary glands. A single larva may carry as many s fifteen tumours, and metastases along the dorsal aorta occur. When minute pieces of the tumours were transplanted into adult flies, all died from extraneous nfection, but in two cases considerable growth of the tumour occurred first. *In vitro* the tumour continues to form pigment. Stark was unable to isolate any organism or virus from the tumours, but found on the contrary that they appeared ust as usual when the rearing of the larvae was done under aseptic conditions. They appeared to be unaffected by X-radiation.

Several years after the first enquiry of Stark, the *lethal*-7 stock was re-examined (Morgan, Bridges & Sturtevant) and the surprising discovery was made that although the gene was still lethal, the tumours had disappeared. Nevertheless he tumour could always be brought out again by crossing with other stocks. Appearance of the tumours, therefore, is not a *necessary* condition of this lethality. In a recent paper, Stark[3] has given improved microphotographs of the tumours, but proposes a theory of their origin from embryonic "rests" which is not ve illuminating. However, when it is remembered that extensive infiltration surrounding tissues from the tumours seems to occur, it is hard to understan why the statement is so often made that cancer does not occur in invertebrates (cf. also the tumours of annelids, described on p. 259).

In 1919 Stark[2] discovered another kind of tumour in *Drosophila* as a mutant, also a melanoma, but not sex-linked and not lethal. It develops in early or late larval stages, and may replace a wing or a leg, but its persistence into the adult stage in abdomen or thorax does not seem to decrease the length of life. Aseptic transplantation to normal larvae did no harm to either; the donors completed their metamorphosis without tumours, and the hosts continued to live with the melanoma within them. The genetics of this strain were subsequently studied by Stark & Bridges.

Still another hereditary melanoma of the fruit-fly was discovered by Gowen[2].* It is sex-linked and lethal to adult flies, which carry it only in their appendages; it does not appear in the pupa at all and is certainly not contagious. This tumour was of X-ray mutant origin; Rapoport[1] describes several further types, all lethal, and similarly produced. X-ray treatment, in the hands of Enzmann & Haskins[1], has produced a fourth melanoma strain, in which the tumour occurs only between the post-alar bristles and varies from a small black button to a large horn-shaped excrescence as big as the fly itself. This tumour, on the other hand, was found to be sensitive to X-rays.† Enzmann & Haskins[2] also found that a persistent larval spiracle may, under the action of X-rays, express itself either as a melanotic tumour (most commonly), or as small wing-shaped structures, halteres, or orific fringed with bristles. Hereditary tumours in butterfly stocks have also bee described (Federley). The hereditary tumours of insects offer a most invitin field for future work.

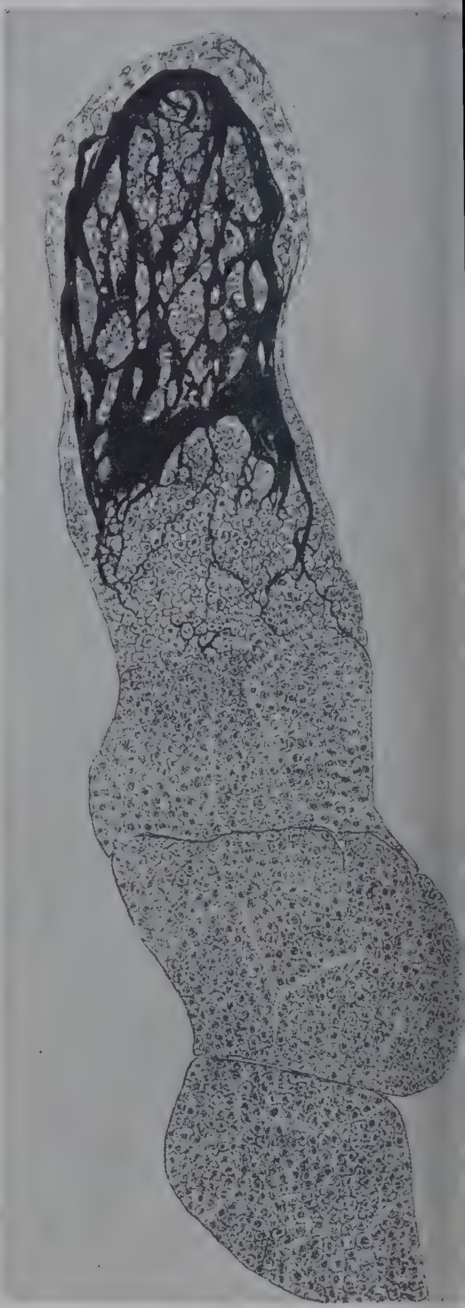

Fig. 229. Hereditary melanoma *Drosophila* imaginal rudiment.

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* In an interesting review, Gowen[3] has related the genetically induced tumours of insects inheritance of resistance to cancer in mammals, and even to inheritance of resistance to infectio diseases. For further information on the benign larval melanomata see Eliz. S. Russell.

† Phenocopies of these melanotic tumours have been produced by Rapoport[2] using As compound

It is not generally known that hereditary tumours have been described in fishes (Reed & Gordon; Gordon; Gordon & Smith). Like those of the fruit-fly, these too are melanotic, resembling melano-sarcoma in their final stages; they arise from a hyperplasia of the epidermal macromelanophores, which later invade the underlying muscles. They may appear at any time in life, but if in the late embryonic stages they are invariably lethal. No metastases occur. The tumour-bearing strains which have been most studied occur on crossing Mexican platyfishes (e.g. *Platypoecilus maculatus*, *variatus*, *xiphodium*, etc.). A review by Kosswig on hereditary tumours in fishes is available, and G. M. Smith in describing a tumour carrying a red pigment in *Pseudopleuronectes*, gives a guide to the plentiful literature on fish "cancers", some of which may be suspected to have a hereditary basis.

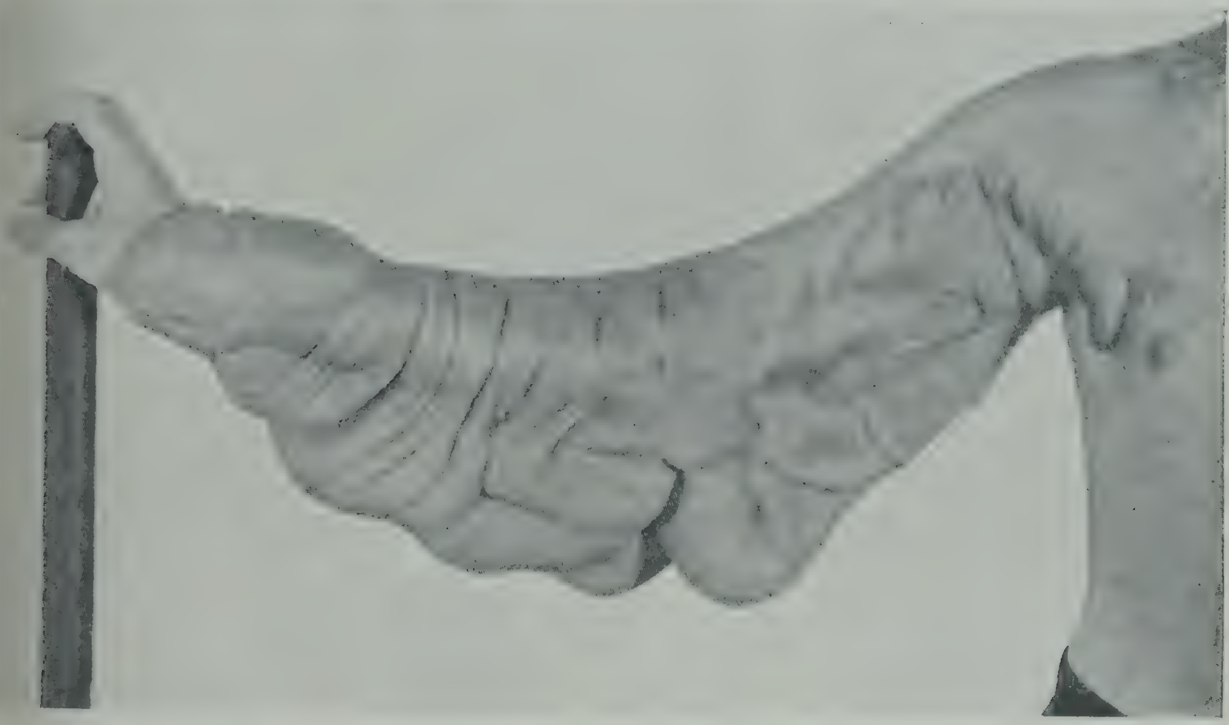

Fig. 230. Neurofibroma simulating elephantiasis (v. Recklinghausen's disease).

It will be remembered that in man also hereditary tumours are known, e.g. the neurofibroma simulating elephantiasis in v. Recklinghausen's disease, for which the short note of Anzinger and pathological books such as that of Bland-Sutton[2] may be consulted (see Fig. 230). Into the whole question of the inheritance of resistance to cancer we cannot, of course, attempt to go here, but the review of C. C. Little[3] will be a help in the exploration of the literature.

2·655. Genes producing anomalies of embryonic metabolism

In a sense all the lethal genes which have so far been described might well be included under this heading, for their effects are undoubtedly accompanied by various metabolic changes, and if they affect organiser action directly, as some of them seem to do, this action is likely to be on the chemical properties or relationships of the inductor substances. But there are cases where a single g has been shown to produce a wide variety of effects, some of which may be sh to be caused by others, but the totality of which is difficult to relate to any com factor. In these "pleiotropic" cases, it is likely that the fundamental anor is metabolic, manifesting itself in different ways.

Two such cases have been given intensive study by Grüneberg. He describ an autosomal recessive gene[3] in the mouse, *grey-lethal*, which suppresses formation of all yellow pigment in the coat and kills the homozygotes at the of 22–30 days. The animals fail to grow normally and die at weaning if a su of finely divided food is not given, but even this cannot prolong their life bey 42 days. Skeletal abnormalities are serious. There is a persistence of spong spicules in the bones, incomplete calcification, and complete retention of teeth[2]. This tooth retention, the consequent reduced use of the masticatory musc etc. ends by distorting considerably the shape of the skull. No connection as yet been found between the disturbances of lipochrome and bone-calcifica metabolism respectively, but later work has given a little further informat Thus Grüneberg has shown that the absence of secondary bone absorption is due to any lack of osteoclasts, and has noted a degeneration of the thymus cor Injections of thymus, however, had no good effect. According to Watch *grey-lethal* mice are characterised by low blood phosphate, low liver glycog and rather low blood sugar, though the muscle glycogen is normal. The mean of all this remains enigmatic.

The other lethal discovered by Grüneberg[4] was in the rat; though not named, we shall refer to it here as *emphysemic* for convenience. An anomal cartilage formation (quite distinct from achondroplasia) affects particularly thoracic skeleton, thickening the ribs, narrowing the tracheal lumen, and p ducing emphysema of the lungs by fixing the thorax in a compressed positi From this central result death may follow by a variety of processes, as shown the accompanying diagram (Fig. 231), taken from Grüneberg. As yet noth definite is known about the anomaly of the cartilage which sets the whole t in motion, but the abnormal cartilage behaves selfwise when transplanted normal body, and *vice versa* (Fell & Grüneberg). In explants the abnormalities not appear.

A very different sort of pleiotropic gene, the effects of which will be even m difficult to unravel than these mammalian ones, is the *dia* gene of the waxm *Ephestia*, discovered by Strohl & Köhler. This involves depigmentation of body, shortening of the length of adult life, and a great reduction in fertility the female moths.

We may now pass to some instances where the effect of the gene seems to h been tracked down with some precision to a definite metabolic anomaly. A turbance of normal water and protein metabolism seems to be involved in action of the gene *sticky* of chicks (Byerly & Jull[1]), a lethal which stops hatchi There is no absorption of the amniotic liquid, which contains much protein, there is no withdrawal of calcium from the shell for the bones, as normally. bones have a certain chondrodystrophic appearance and calcification is dela

(T. P. Sun). The gene is recessive, not dominant, like *creeper*. According to Ogorodny & Penionskevitch vitamin-B_2 deficiency is connected with this anomaly.

Disturbances of keratin metabolism seem to be involved in the action of another chick gene, *frizzle*, which has received much study at the hands of Landauer[5] and his school.

Frizzle in the homozygous condition was proved by Landauer & Dunn[1] to be lethal in 1930, though this had previously been suspected. The heterozygote forms the long-known Frizzle breed, of wide geographical distribution. *Frizzle* feathers are curled and quite abnormal in appearance; the homozygote has none at all for the first year of its life, and though later it grows a sparse plumage it does not usually reach sexual maturity. Determination of the anomaly is evidently complete by hatching, for skin transplants to normal chicks always behave selfwise (Landauer & de Aberle). When the abnormal physiology of *frizzle* chicks was analysed, it was found that the excessive loss of body heat causes a striking increase of the basal and general metabolism (Benedict, Landauer & Fox). Although *frizzles* show a large increase of food-intake and reduced rate of water-loss, they usually cannot balance their metabolic requirements. Correspondingly, the *frizzle* heart rate is raised much above the normal (Boas & Landauer[1]) and so is the actual weight of the heart (Boas & Landauer[2]). The

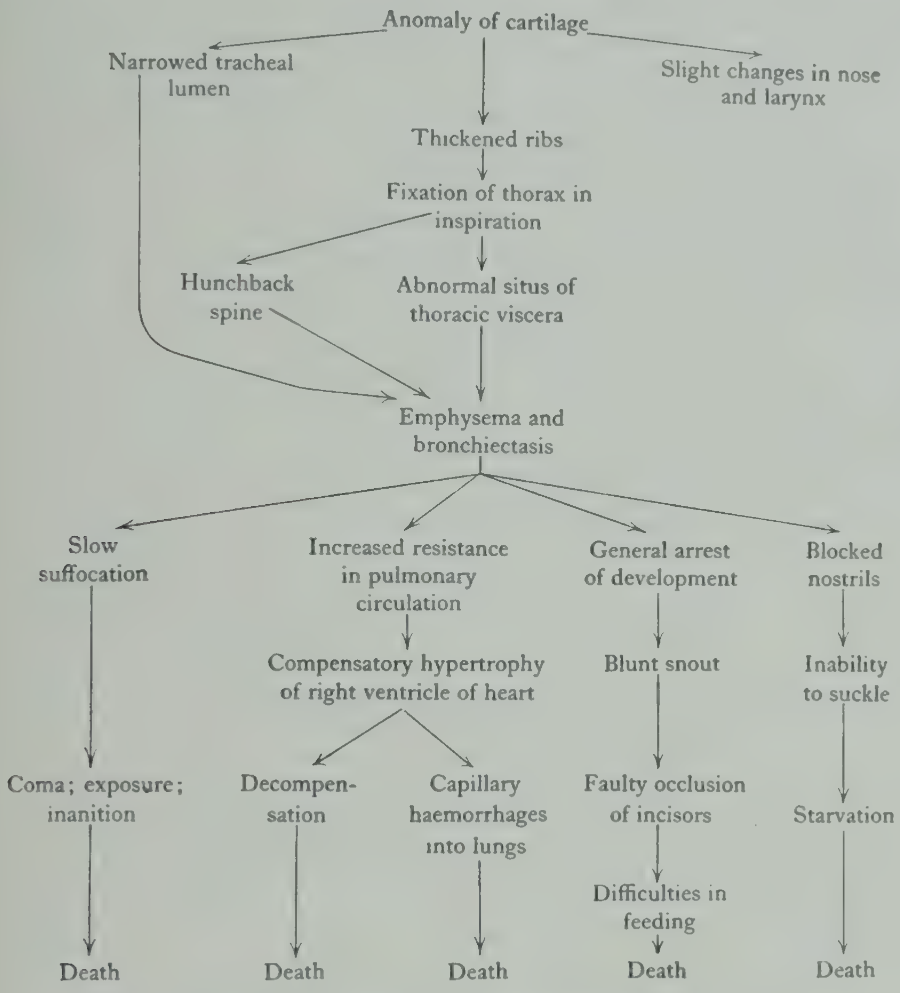
Anomaly of cartilage
Narrowed tracheal lumen
Slight changes in nose and larynx
Thickened ribs
Fixation of thorax in inspiration
Hunchback spine
Abnormal situs of thoracic viscera
Emphysema and bronchiectasis
Slow suffocation
Increased resistance in pulmonary circulation
General arrest of development
Blocked nostrils
Compensatory hypertrophy of right ventricle of heart
Blunt snout
Inability to suckle
Coma; exposure; inanition
Decompensation
Capillary haemorrhages into lungs
Faulty occlusion of incisors
Starvation
Difficulties in feeding
Death
Death
Death
Death
Death

Fig. 231. Multiple effects of the *emphysemic* gene in the rat.

condition, in fact, resembles Graves' disease (Landauer[20]), though the abnorma is peripheral, and this resemblance extends to the blood picture, which studied by Landauer & L. T. David[1]. The position of the thyroid gland in *frizzle* state is complicated (Landauer[13]; Landauer & L. T. David[2]; Landaue Landauer & de Aberle), it may be secreting too little thyroxin or too mu depending on environmental temperature, and it is generally hypertrophi A very complete study of the weights of all organs in normal and *frizzle* chi at different ages has been made by Landauer & Upham; it agreed with w would be expected from the raised basal metabolism of the *frizzles*. Fina *frizzle* chicks show peculiarities of the temperature-regulating mechani (Landauer[22]); this is a secondary effect of the semi-naked condition.

The thyroid gland of *frizzle* chicks undergoes a compensatory hypertrop But other genes are known which seem to have a direct metabolic effect on production of a particular hormone. Such is the gene for pituitary dwarfism mice, first discovered by Snell[1] in 1929 and studied by Smith & McDowell. T growth curves of *dwarf* mice show an enormous lag behind the normals, a according to Dawson[1] the retardation of skeletal ossification is such that the dw takes six months to reach the stage attained by the normal in six weeks. Sm & McDowell first showed that by transplantation of normal pituitary glands, growth of the *dwarf* mice could be restored to normal, and they then carried analysis further by demonstrating that in the pituitaries of dwarfs only growth hormone was missing, not the gonadotrophic.* Hereditary dwarfism known also in the rat (Lambert & Sciuchetti), but here the mechanism seems be more obscure as pituitary treatment did not restore growth to normal. It known also in the rabbit (Greene, Hu & Brown) and in the guinea-pig (Sollas

Enzymes as well as hormones may be specifically suppressed by genes. Th Glembosky found the essential cause of a homozygous lethal's action in caracul sheep to be the absence of gastric rennin.

Lastly, the peculiar cases of hereditary muscular contracture or tremor m be mentioned as probably occasioned by a genic interference with mus chemistry. Hutt has described a gene causing muscular contractures and death of calves at birth, and a recessive lethal causing 85 % deaths with viol muscular tremor after hatching exists in chicks (Hutt & Child). Even in *Drosoph* a *shaker* strain has been described (Lüers). Cole & Ibsen's congenital palsy guinea-pigs presumably belongs here too.

That quite subtle metabolic differences are affected by genes is of course w established. Avoiding eye- and coat-colour examples as somewhat hackney mention may be made of the *Drosophila* strain found by l'Heritier & Teiss

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[*] Prolactin and the thyreotrophic will act instead of, or perhaps because they *are*, the gro hormone here (Bates, Laanes & Riddle). The Californian school nevertheless maintains absolute separate existence of the growth hormone (Evans; Meamber, Fraenkel-Conrat, Simp & Evans). Others, e.g. the Montreal school, have suggested that the pituitary hormones may be prosthetic groups of the same protein.

[†] Perhaps the most beautifully analysed case of hereditary dwarfism is in plants, not anim Van Overbeek found that the gene *nana* in maize produces dwarfism by causing excessive per dase activity, which is associated with abnormally great auxin destruction, and hence d growth.

which was sensitive to pure CO_2. In general the gas has only a reversible anaesthetic effect on flies, but in this strain the damage done by it was irreversible. Similarly, Lamoreux & Hutt have discovered genetic susceptibility to vitamin-B deficiency in the chick. But to pursue this subject further would be to leave the realm of lethal factors interfering with morphogenesis.

2·656. Genes killing or deforming the embryo by unknown mechanisms

Into this category fall perhaps the majority of lethal genes at present known. Many lethals in *Drosophila* have been reported (e.g. in the papers of N. P. S. Dobzhansky; Kaliss; or J. C. Li[1], or in the monograph of Morgan, Bridges & Sturtevant), but we understand practically nothing about their mode of action. Or we might take as examples the lethal genes in the grouse locust *Apotettix eurycephalus* described by Nabours & Kingsley. Some of these embryos develop normally till four days before hatching, and then die showing no irregularities or deformities whatever. The same sort of mystery surrounds the gene *hooded-lethal* of mice described by Crew & Kon. This recessive gene kills the newly born mice in the second week of lactation by inanition, but no causal mechanism has been found. In birds, too, lethals of unknown action have been described. Dunn[1] found a lethal gene linked with recessive white plumage in Wyandotte fowls which destroys the embryos in the first week of development. Upp & Waters suspect the existence of a sex-linked one in White Leghorns.

It is evident that the field of the embryology of lethal genic action offers a vast scope for work on "reversed" experimental embryology, particularly hopeful owing to the recent advances in experimental embryology itself, and the aid which biochemistry is beginning to be able to give.

2·66. The kinetics of gene action

With the subject of this division we find ourselves right in the middle of the debatable land between embryology and genetics. Those who have no training in the niceties of genetic analysis may be forgiven for abiding in their own regions of biochemistry or morphology as the case may be, but nevertheless some account of the mechanism of action of genes in embryonic development is indispensable in a book of the scope here planned. Readers who wish for more information will of course find it in the standard works on genetics.*

Chemical embryology is involved here for two reasons. In the first place, some chemical reactions at the phenotypic end of the chain of gene causation are fairly well known. But often, as in the case of pigment production, these may not be very interesting from the morphogenetic point of view. Secondly, the effect of external agencies, such as temperature change, on the development of the phenotype, has shown that the intermediate processes in the chain of gene causation are themselves chemical reactions. If the end-result is a morphological change this fact is therefore very interesting. We shall begin by discussing the s point and finish with the first one. One realises to-day that the developm an organ or part is a complex of processes presided over at every succ stage by the appropriate genes. Perhaps the fullest illustration of t Waddington's work[23] on wing-differentiation in *Drosophila* in which s separate but not necessarily independent processes can be distinguished, the control of some forty different genes.

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* It may be repeated at this point that in these matters the present book expressly disclaims any pretensions to being an exhaustive examination of the relevant literature.

2·661. Phenocopies and rate genes controlling morpholo processes

It has already been mentioned that changes in the phenotypic end- brought about by genetic changes may be imitated by externally applied age or by spontaneous factors normally occurring. Such effects may only be tinguishable from the genetic effects by the fact that they are not inherited. previous section has afforded a number of examples of these *Phenocopies*, a following table shows:

Genetic effect	Phenocopy
Hereditary otocephaly (Wright)	Spontaneous teratological otocephaly
Hereditary pseudencephaly (Snell)	Failure of neural tube to close in the absen sufficient mesoderm (Holtfreter)
Hereditary rumplessness (Landauer)	Rumplessness due to abnormal temperatures (Danforth)
Hereditary failure of cornea induction in Bagg-Little strains (Bonnevie)	Failure of cornea induction in blind cave (Schlampp)
Hereditary aristapedia (Balkaschina)	Aristapedia produced by boron (Rapoport) Regenerative aristapedia (Brecher)
Hereditary melanoma (Stark; Gordon)	Melano-sarcoma
Hereditary achondroplasia (Landauer)	Experimental achondroplasia (Fell & Landaue
Hereditary wing pattern (Goldschmidt)	Experimentally produced wing pattern (Goldschmidt)

The last entry in this table leads us from the study of lethality and its deep-se dislocation of embryonic processes to those lesser changes within the borde the "normal" which are the study of geneticists.

In the nineties of the last century it was found by a number of workers Dixey; Standfuss) that the wing pattern of butterflies can be changed by action of abnormal temperatures during the pupal period in such a way tha experimentally produced pattern cannot be distinguished from that of other of the same species. Thus Central European *Papilio machaon* pupae subjecte unusual temperatures produced butterflies almost indistinguishable from Syrian *Papilio sphyrus* or the *Papilio centralis* of Turkestan. At that t owing to the influence of vague evolutionary speculations, the real signific of the effect was not noticed,* and it was left for Goldschmidt[2] in 1917 to p out that as temperature might most reasonably be expected to influence of chemical reactions, it was probable that the action of genes in produ patterns was due to their influencing such reaction rates. This early work not confined to insects. Beebe had found in 1907 that changes in the feathe of doves such as *Scardafella inca* could be produced by the action of unusual humidities during the early post-hatching period, shifting the appearance of the animal towards types of more tropical origin.

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* Spemann's remark, made in another context, would fit here: "Man kann eine Entdec machen ohne es zu wünschen, aber doch nicht ohne es zu wissen."

Goldschmidt[6] later on made a systematic investigation of the phenocopy phenomenon in *Drosophila*, using high temperatures applied at different times during development. In this way phenocopies of very many known mutations were produced, such as bristle changes, wing changes, and even the production of benign tumours. In every case the exact conditions for producing a large percentage of a given phenocopy are now known. They include the following principal factors: (1) the developmental stage at which the abnormally high temperature is allowed to operate, (2) the time during which it is applied (6–24 hr.), (3) the exact temperature (35–37° C.), (4) the genetic constitution of the "normal" (wild-type) animals used. It has since been shown that exposure to abnormally low temperatures (Gottschewski), to X-rays (Friesen) and to high-frequency electric current (Malov & Friesen), will similarly produce phenocopies, but naturally they are not the same ones, and the exact conditions vary.

That rates of reaction now occupy the centre of the picture is not remarkable in itself, since any interpretation of the facts of embryology must contain the time variable, however disguised. But the essential point is that the normal integration of all the reaction rates in the embryo is subject to interference. Such interference may be brought about by external agencies at the will of the experimentalist, or by internal genic change. Thus in a former section, we had occasion to notice with special interest a case in which the onset of a determination process was shifted with relation to visible morphological differentiation by the action of temperature (Twitty, see p. 337). Later on we shall examine a number of other cases which demonstrate the "dissociability of the fundamental processes in ontogeny" (p. 505). That genes are catalysts or producers of catalysts (enzymes) or producers of inhibitors is a doctrine now generally accepted, largely due to Goldschmidt's advocacy[8]. And if gene changes shift the rates of reactions during embryonic development relatively to one another, so also can external agencies, particularly changes of environmental temperature. In this sense all genes are "rate genes".

A caution might perhaps be interpolated here. Wright[5] and his collaborators, for example, in analysing their remarkable series of gross hereditary morphological anomalies of the head, have tended to use the concept of rate genes to explain the distortions, suppressions, etc. as due to inhibitions of "developmental rate" at various moments of development. But this is a relatively vague notion, much vaguer than that of a definite chemical reaction such as the production of a pigment from a chromogen. And it has in fact been unsatisfactory in so far as it has left out of consideration the primary and second-grade organisers of the head, i.e. certain chemical substances, some of the properties of which we already know. When an evocator acts from an inductor upon a competent reactor there are several types of reaction to be considered; that which synthesises it chemically; that which liberates it from an inactive precursor; that which conveys it to its destination whether by diffusion or otherwise; and finally those which it itself sets in motion or catalyses. In addition to these, there are the actual differentiation rates of the inductor and reactor tissues. A Stockardian or Child "developmental rate" is unsatisfactory, because it confuses all these thi

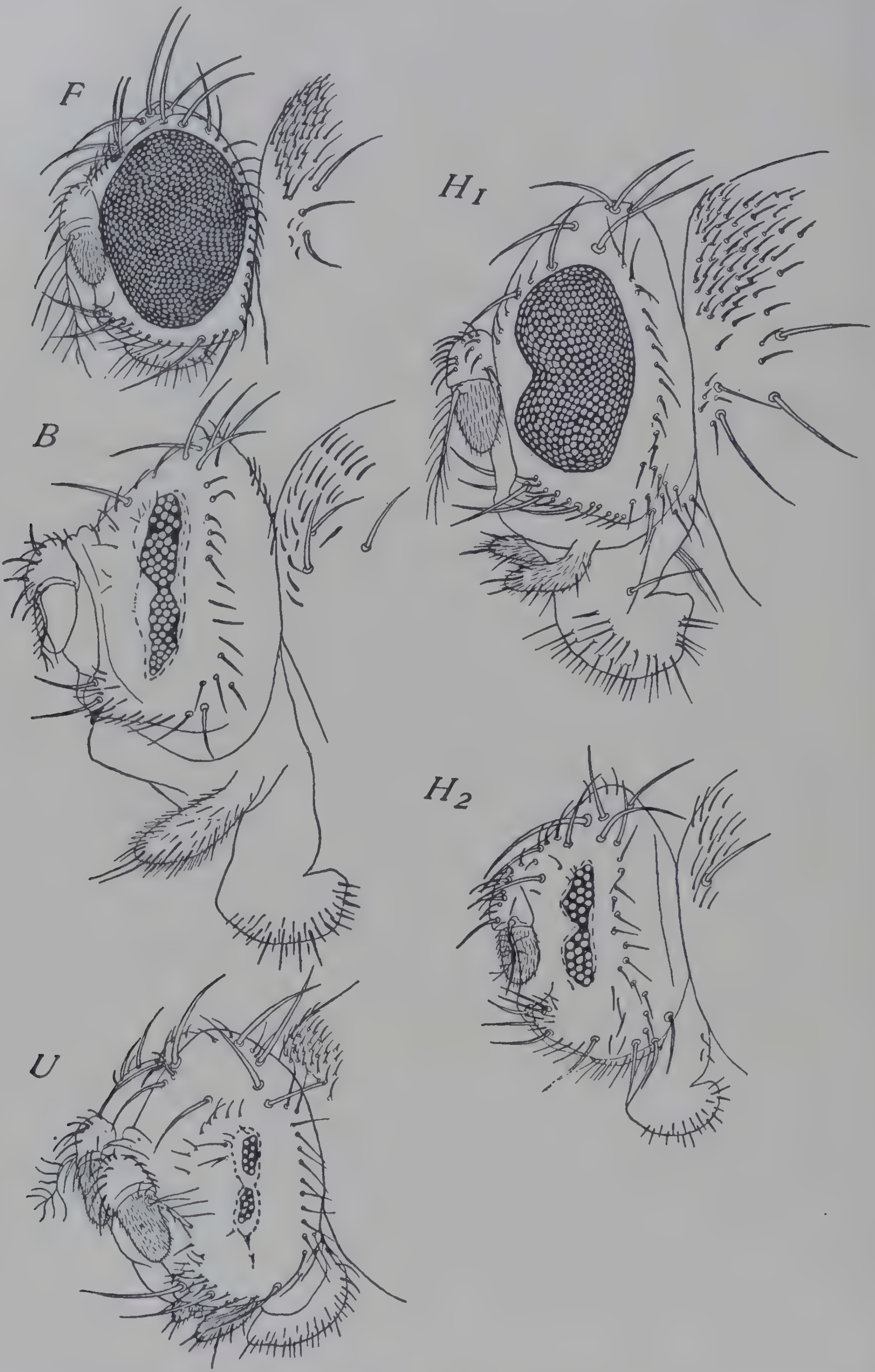

Fig. 232. Effects of *bar* genes in *Drosophila*. *F*, full-eye wild type; *B*, white *bar*; *U*, *ultrab* H_1, heterozygous F/B; H_2, heterozygous F/U.

together. The inhibition of an organiser action is a quite definite concept, a the nature of such inhibition (of which we have already seen several exampl cf. pp. 223 ff., as well as Wright's otocephaly) demands specific study. But rates of reaction will ultimately be involved none the less.

Controlled environmental temperature can not only produce from a given type (the "wild type") of organism, phenocopies presenting the characteristics of other types (e.g. geographical races); it can also produce phenocopies of quantitatively different members of a series of multiple allelomorphs. Of this the *bar* gene in the fruit-fly is the classical example. As is well known, the fruit-fly has a series of genes all producing more or less reduction in the number of facets in the eye (a morphological but quantitative effect). These effects are shown in Fig. 232, taken from Zeleny's account[2] of *ultrabar*, and in Fig. 233 (opp. p. 372) by some photographs recently published by Kikkawa[2].

The *bar* gene was originally discovered by Tice in 1914 and soon afterwards Zeleny[3] and his collaborators began a systematic study of it. Thus, for example, the following reductions of facet number occur at 27° C. in the female:

	Average number of facets present
"Wild type"	810·6
Infrabar	362·0
Bar	61·8
Ultrabar	22·0

Seyster and Krafka then found that the number of facets in the eyes of flies of known genetic composition varied in accordance with the temperature at which they were cultivated, and that the higher the temperature, the fewer facets were formed. Thus it appeared that for every increase in temperature of 1° C. the number of facets decreased by 10 %. From Fig. 234, taken from Hersh's work[1], it can be seen that the relationship is linear on the semi-logarithmic plot, and that a *bar* fly at 30° C. has as few facets as (i.e. is a phenocopy of) an *ultrabar* fly at about 18°. The homozygous state is also, of course, a factor; thus homozygous *ultrabar* at 15° has the same number of facets as the heterozygote at 25°. Such relationships are known as *Thermophenes*.

A considerable literature has arisen in the detailed study of the *bar* allelomorphs; it may be explored through the papers of Zeleny[3]; Hersh; Luce; Driver; Margolis & Robertson, etc. Here we only need to observe that the action both of increased temperature and of the various genes of the series must be to accelerate differentially some chemical reaction inhibitory of the facet-forming mechanism. Again the parallel with the case of determination of ciliary polarity should be drawn, for there (p. 337) increase of temperature delays the onset of the determination relative to the morphological age.

It seems fairly clear that until we know much more than at present about the nature of the facet-forming mechanism itself, speculation about the action of the *bar* gene series or temperature, and even calculations of temperature coefficients and characteristics on various assumptions, will not be really illuminating. The possibilities are numerous. We do not even know how far inductor effects are involved, and as Margolis has pointed out, the nature of the facet-inhibiting reaction might be a removal of the facet material, e.g. by prior determination in other directions. The only relevant study of the necessary embryology is co tained in the careful work of T. Y. Chen, who measured the sizes of the imagin buds of the eye and other structures in the larvae of normal and mutant flies The imaginal buds for the eyes occupy a position in the larva in the metathorac segment just posterior to the antennal buds and anterior to the larval gangli

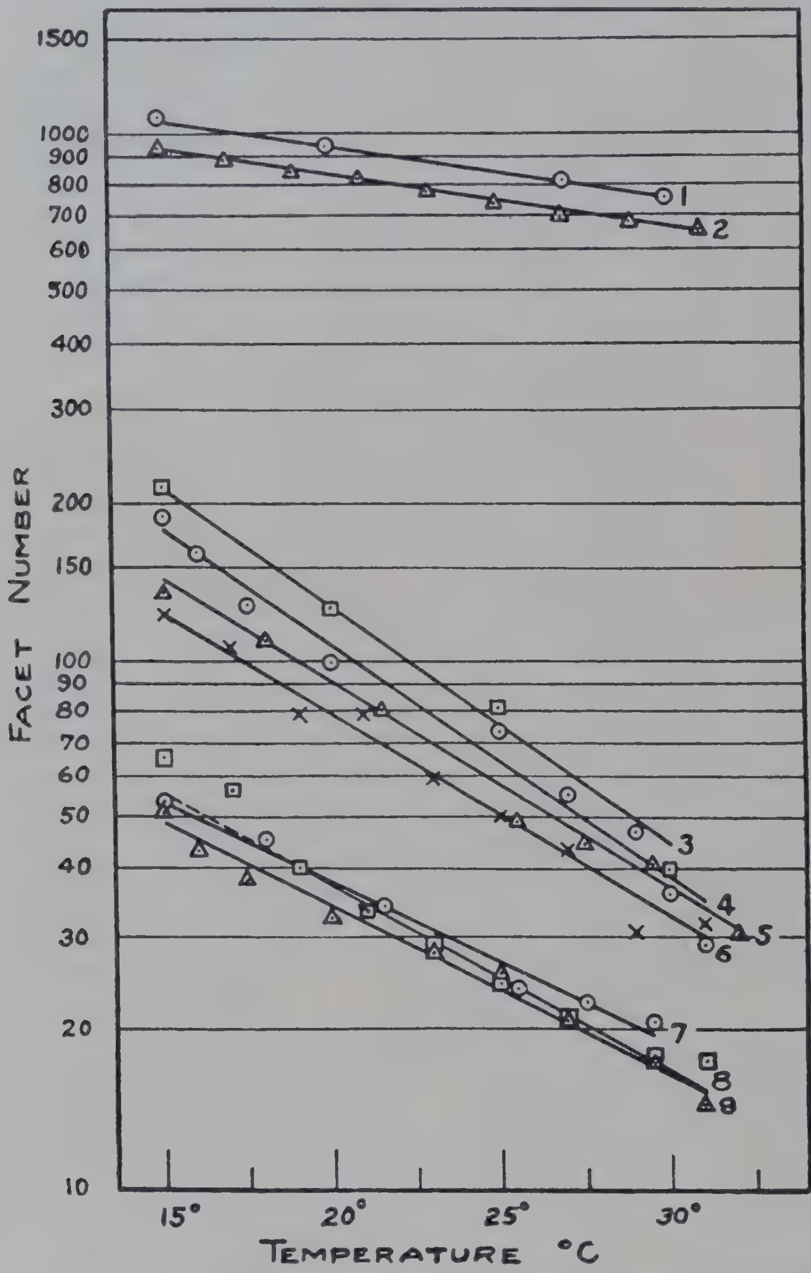

Fig. 234. Thermophene of *bar* eye *Drosophila*. 1, Wild type full; 2, reverted full; 3, unselected *bar*; 4–6, different low selected *bar*; 7–9, various *ultrabar*.

The presumptive facet region is distinguishable in them from a very early tim As the following table shows, the eye buds of the mutant types in which eye si and facet number are reduced, are affected from the earliest time at which the sizes can be measured.

* The elaborate work of F. Bernard on the quantitative embryology (heterauxesis of facets, et of arthropod eyes, should not, however, be overlooked.

	Size of the optic buds in sq. μ	
	48 hr. larva	Mature larva
Normal ("wild-type")	128	535
Lozenge-3 (a gene causing facet-fusion and giving the eyes a glazed appearance)	—	360
Bar	—	232
Eyeless-4 (suppressing eyes in adult)	11	107

In the two last cases other imaginal discs, such as the antennal buds, were demonstrably of normal size, so that the effect is very discriminatory. Chen had some reason for thinking that in the earliest stages the eye buds of *bar* were relatively unaffected, and that the inhibiting influence came on later. This is important in view of the later work of Goldschmidt[6] on *vestigial* wing forms (see below, p. 400).

Chen's work was later amplified by Medvedev[1], who studied the mutant races *lobe-c*, *glass*-2 and *eyeless*-2 which are all characterised by various degrees of reduction of eye size. In addition, the first and third of these genes produce asymmetry in eye suppression, so that one side of the head may have no eye while the other side may have a more or less well-developed one. The results of Medvedev's measurements, summarised in Fig. 235, showed that as early as the 24th hour the differences between the types were visible, but become more pronounced as time goes on. The drop at the 8th hour is due to the fact that before that ime the whole cephalic complex (eye bud nd antenna bud together) was measured, nd after that time the eye buds only. The arval ganglion in the three races and the vild type served as a control; not a trace of lifference in size or growth rate was observble in it. It remains to be decided whether nhibition of cell-divisions or some other nechanism is the basis of this hereditary size limitation of eye buds in mutant *Drosophila* races.

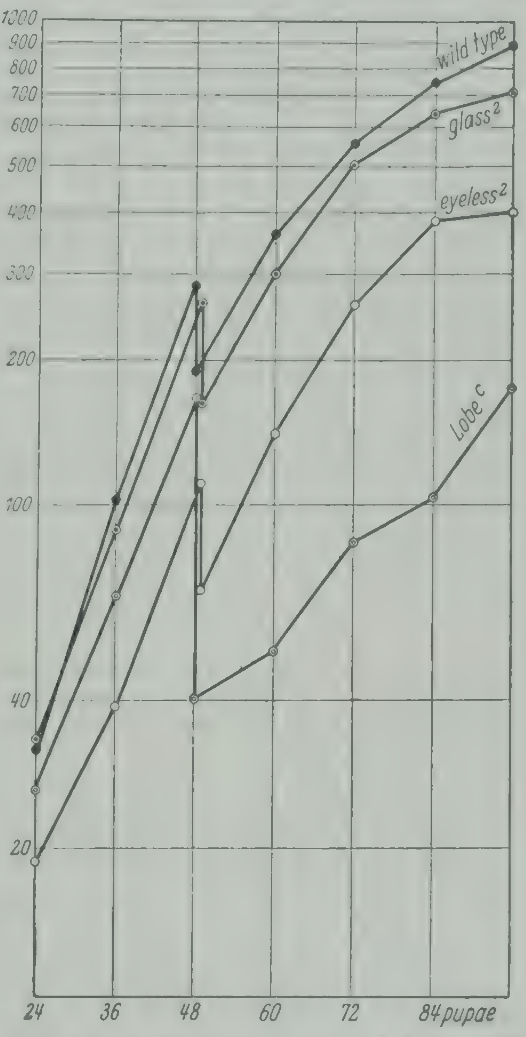

Fig. 235. Logarithmic curves of the growth of the imaginal discs of eye mutants.

Further light on the production of eye abnormalities by genes in arthropods comes from the work of Wolsky & Huxley[2], who, starting from the fact that in he non-faceted regions surrounding a *bar* or *ultrabar* eye there is a deposition f the usual eye pigment, showed that in these regions ommatidial (sensory) cells xist, but in an atrophied condition. As normal facets only occur in the cuticle over normal ommatidia, there is a presumptive parallel with lens inductior vertebrates. The *bar* genes would thus interfere with "eye-cup inductio (cf. avitaminosis-A, referred to on p. 227, or the otocephalic syndrome, p. 37 On the strength of this analogy Huxley[14] rectified the interpretation previou current of Sturtevant's interesting mosaic experiments. Sturtevant[2] found t if a mosaic spot of genetically wild-type tissue happened to occur in the reg of a *bar* eye, the number of facets was considerably increased as compared w usual *bar* eyes, as if the wild-type tissue had induced facet formation in adjac *bar*-type areas. Huxley pointed out that as the number of ommatidia was increas as well, the increase in facet number must have been secondary, and the ba effect of the wild-type tissue must have been upon the growth rate or determi tion of the ommatidia in the eye bud at earlier stages.

Parallel investigations have been made on the development of the normal a mutant eye in the shrimp *Gammarus* (Schatz; Wolsky & Huxley[1]). Some ra (e.g. *red*) differ from the normal only in pigmentation (see below, p. 407); oth have seriously deficient eye structures (e.g. *colourless*).

If facets are really induced by ommatidia, the existence of a facet-formi substance would be expected. Now transplantation experiments by Beadle Ephrussi[2] (see below, p. 408) on *Drosophila* had shown that *bar* eyes, contrary wild-type eyes, do not produce the "vermilion substance" required in the pigme formation of wild-type flies. Thinking therefore that the pigment-formi substance might be related to the facet-forming substance, Ephrussi, Khouvi & Chevais supplied *bar* larvae with extracts (prepared from *Calliphora* larva rich in the former. The results were astonishingly clear; as the following tab shows:

	Average facet number
Control *bar* fly	103
With 4 % extract	144
With 8 % extract	244
With 10 % extract	455

A morphogenetic substance under genetic control is therefore probably at wor

That this is independent, however, of the pigment-forming substanc was subsequently demonstrated (Steinberg & Abramovitch; Chevais, Ephrus & Steinberg). Eye discs from *bar* larvae which had been fed a *Callipho* facet-forming extract were implanted into *vermilion* hosts. Such facet-increas *bar* eyes then developed the same pigmentation as eyes from untreated *bar* larv (containing many fewer facets) similarly transplanted. Facet number can th clearly be affected without affecting the colour eventually attained by the ey and the facet-forming or reducing reaction system must be regarded as indepe dent of the pigment-forming system.

The other instructive cases of the interaction of environmental temperatu and genetic constitution take us away from the eye, to the suppression of bristl and wings. Probably the first mathematical analysis which the phenomen received was that devoted to the fruit-fly gene *dichaete* by Plunkett. This ge removes certain bristles from the fly's thorax, especially the dorso-central one Just as in the case of *bar* eye, increase of temperature was found to intensify th effect, although the points seem to need not one straight line on arithlog paper but two meeting at an intersection about 25° C. (Fig. 236). Plunkett proposed that there might be an increase, relative to other developmental processes, in the rate of a reaction destroying a bristle-forming substance or forming a bristle-suppressing substance. He was able by calculation to reach the conclusion that the primary effect of the *dichaete* gene was the destruction of an enzyme. But in the absence of any experimental and biochemical evidence these conclusions must remain to some extent hypothetical.* The analysis has since been much refined by G. Child.

When the effects of genes reducing wing size in the fruit-fly were studied, exactly the opposite relation with temperature was found. Roberts[1], and following him, a succession of investigators, showed that increase in temperature during the larval stage of *vestigial* flies increased the length of the wing retracing all the steps towards the normal wild-type wing. Fig. 237, drawn from the data of Harnly and Riedel, shows that a sharp increase in length takes place just below 30°. At lower temperatures the breadth is more affected. Wings of wild-type flies, on the contrary, diminish in length with rising temperature, but to a much lesser extent.†

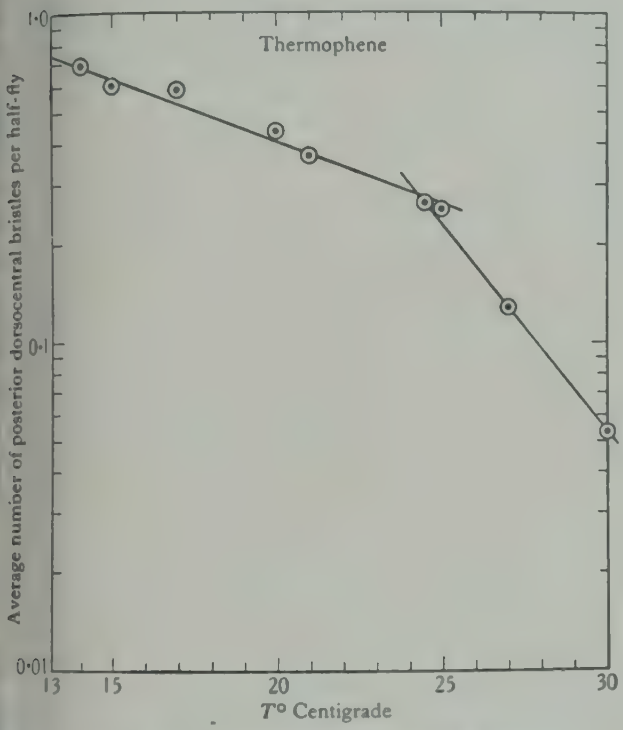

Fig. 236. Thermophene of *dichaete Drosophila.*

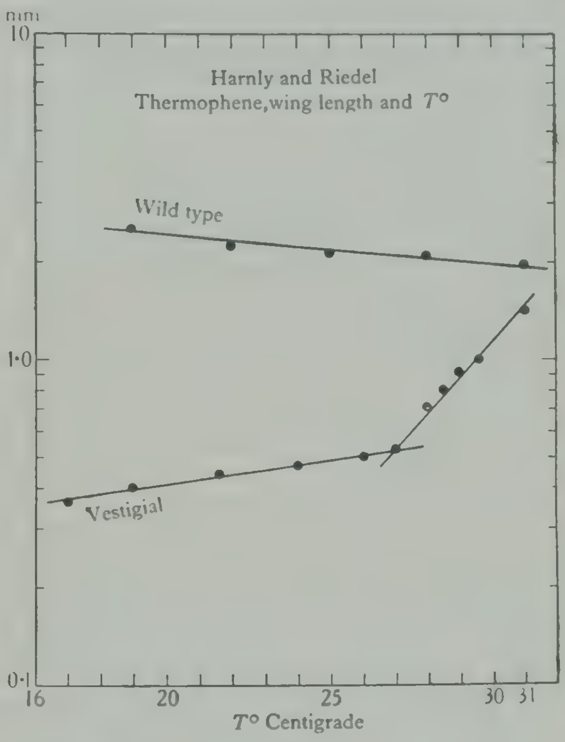

Fig. 237. Thermophene of *vestigial* and normal *Drosophila.*

The embryology of wing reduction in the allelomorphic series *vestigial* was studied by Goldschmidt[6] with remarkable results. The action of the genes of this series ranges all the way from the normal wing through *notched*, *nicked*, *ragged*, *antlered* and *strap* to *no-wing*. The various stages of the series show greater and greater reductions of wing area starting from the tip and going on until nothing but a stump is left. These reductions take the form at many stages of a scallopi i.e. a more intense reduction of areas between the veins. This is illustrated in 238 by a topographic model based on the data of Goldschmidt (*Physiol. Genet* p. 229), where the base represents the normal shape of the wing and the summit the shape of the most highly reduced wing. Goldschmidt now found that the development of the wing proceeds normally at first, but after a certain time degeneration sets in, and a greater or lesser area of the wing is resorbed. The earlier the process begins, the more complete is the abolition of the wing; where *no-wing* is concerned for instance, it begins already in the imaginal disc. It was also found that similar degenerative processes occur in other genetic wing abnormalities, such as *dumpy*, *miniature*, *beadex*, etc. Goldschmidt concluded that the genes either bring about the failure of some necessary substance after a certain time, or else actually produce some "lytic" or inhibiti substance. Whatever the process, it can be modified by the presence of oth genes (Harnly & Harnly).

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* Phenocopies of bristle reduction have been produced by Rapoport using Hg compounds.

† See also Hersh & Ward and Li & Tsui.

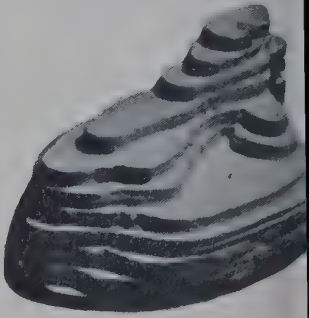

Fig. 238. Topographic mo illustrating the stages wing-reduction in the *ve gial* allelomorphic serie *Drosophila.*

This kind of secondary degeneration of an embryonic structure is not confin to insect development; we have already seen similar cases in mammalian leth (p. 376). Besides the failure of a second-grade organiser for an appendage, a the eyes of cave forms (p. 225), therefore, we have to reckon with histogene failure followed by rather obscure resorptive processes.

Goldschmidt[7] has summarised these relationships* in a family of curves rep senting the growth and decay of all the various quantitatively different allelomorp But it may also represent the production rate of an essential "growth substanc such as a vitamin, in the absence of which development will cease and resorpti occur. Or conversely, it may represent the effects of the production of a ly substance, of localised specific toxicity. Although inclined to regard the seco alternative as the less likely of the two, Goldschmidt drew attention to a rath striking analogy which would exist between the decay of the wings in the *vestig* allelomorphs and the destruction of a population of bacteria by bacteriopha Where bacteria and phage are present together both are increasing ("growing but the phage is increasing faster than the bacteria. When, therefore, a cert phage/bacteria ratio is reached, known as the lytic threshold, a rapid lysis of t bacteria begins and continues till all are destroyed. This threshold can be shifted the will of the experimentalist, e.g. it varies according to the presence or absence traces of manganese. Hence the bacterial growth curve corresponds to the develo ment of the wings of the insect, and the lytic threshold corresponds to the po of maximum development reached by any given mutant wing before it begins degenerate. This we might call the *vestigial* threshold. Just as the lytic thresho can be changed by external factors such as metallic ions, so the *vestigial* thresho can be affected by temperature, for, as we have seen, the higher the temperatu the larger the eventual wing, and so the higher the threshold must have been

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* Other workers, it is fair to say, do not interpret them as Goldschmidt does—see the rec paper of Waddington[23].

It may be useful here to state the threshold concept, which is much used in genetical work (cf. Goldschmidt[8], p. 65), in embryological terms. As long as the amount of an inductor (hormone, organiser, evocator) is below a certain level, no effect will be produced (cf. Fig. 45). But conversely, when the competence of the reactor has been completely implemented, no further increases in the amount of the inductor will have any effect. Thus in the present instance, no increase in the growth substance, or decrease in the lytic substance, will give rise to wings larger or better developed than the wild-type wings (except the wings of related geographical races, which may be slightly larger than those of the type chosen as "wild type").

Goldschmidt[8] thinks, probably rightly, that the threshold concept may explain one of the most puzzling of the effects of the *vestigial* allelomorph series, namely the fact that not all these genes show the same statistical uniformity in their results. Some affect only a small percentage of the animals carrying them, others affect all. The following table, taken from Mohr[2], illustrates this; it reads from the least to the greatest effect:

	Arbitrary numerical wing-suppression factor	Percentage flies showing the effect
Wild type	40	—
Nicked-notched	37	0·2
Vestigial-no-wing	36	1·3
Nicked	32	27·1
Notched-notched	30	42·4
Nicked-no-wing	28	70·7
Notched	25	100
Notched-no-wing	21	100
Vestigial	20	100
No-wing	16	100
No-wing-no-wing	12	100

In this particular case, the weakest genes are also the least certain in their action, but this relationship might not always hold. In order to distinguish between these two phenomena, Timofeev-Ressovsky introduced the terms "penetrance" and "expressivity". Penetrance means the extent to which every animal carrying the gene is affected by it (percentage phenotypic effect). Expressivity means the extent of the effect itself. Thus *nicked* has a relatively mild expressivity and low penetrance, while *no-wing* has a severe expressivity and full penetrance.

Goldschmidt's detailed explanation for the wing-reduction series is that in the minor degrees of wing reduction the *vestigial* threshold is very high, and hence rather late in development. Hence in many individuals the dangerous period might safely be passed before the reduction in the amount of growth substance (or the appearance of appreciable amounts of lytic substance) had had time to become serious. Hence the low penetrance. Where the expressivity is high, the differentiation processes must have been interfered with at much earlier stages, and the dangerous periods must have been much longer; hence the statistically higher penetrance.

This point of view is perhaps supported by the circumstance that we have already met with some facts, not connected with genetics at all, which seem to fit in to the framework of penetrance and expressivity. If reference be made to Fig. 94, it will be seen that in general the effect of a given hydrocarbon in neural induction in amphibia is proportionally upon the number of neural tubes p duced in a series *and* the average volume of the neural inductions so produc Only one hydrocarbon was found (3:4-benzpyrene) which, under the sa conditions as all the others, consistently gave rise to a rather large number relatively small neural tubes. In other words, its expressivity was mild but penetrance was high. The other hydrocarbons of course showed regula increasing degrees of penetrance and expressivity. We have therefore to recogn that in the production of a distinctively morphogenetic effect by pure chemi substances there may be a dissociability between these two forms of biologi reaction. And this would be in general agreement with the view that the act of the genes is mediated by chemical processes whether of enzymic or hormo nature.

Here we cannot go further into the genetics of penetrance, etc., but am information is available in the genetical literature.

2·662. Phenocritical periods

Throughout the foregoing discussion, the effect of environmental temperatu in producing phenocopies, etc. has been considered as acting at all stages development. But it was early suspected that some stages of development wou be more sensitive to temperature than others, and this proved to be the ca The stimulus of raised temperature has to be applied during a relatively sh period of larval life preceding pupation. Exact data are now extant for t (Driver on *bar* eye reduction and facet suppression; W. F. Stanley on *vestigial* wi reduction). The sensitive or "phenocritical" period for *bar* begins at the ti when the cephalic complex is separating into the eye bud and the antennal bu and ends about the time of formation of the first facets. The sensitive period *vestigial* begins at the third instar (larval moulting period) when the wing bu are just being formed. Now as rise of environmental temperature can, as have seen, counteract the wing-reduction process (either by differentially increasi the rate of a reaction furnishing growth substance or by differentially increasi the rate of a reaction destroying lytic substance), it must take effect actually the larval wing buds. Phenocritical periods and thermophenes have also be carefully delimited for *scute* (a bristle-reducing gene in *Drosophila*) by G. Chi for *short-wing* by Eker, for *mottled-eye* by Surrarrer, for *eyeless* by Baron, etc. e

With the phenocritical period we can link up what has been said in earl sections regarding lethality (p. 365) and competence (p. 116). The *vestig* allelomorphs represent a series of genes the effects of which go farther and fartl back into development in accordance with the severity of their final effec *No-wing* in the homozygous condition is actually lethal. It is lethal becaus gives rise to great reductions in the size of the thorax, and it does this becau it affects the wing rudiments at a time so early that they have not as yet beco separated from the thorax segment material, also contained in the same imagi disc. If a gene acts sufficiently early in development, therefore, it will aff tissue still in a highly competent state, still to a large extent undetermined; a hence the dislocations which it produces will be all the more severe. In *vestigial* series, only those genes which have effects less severe than *snipped* are affected by temperature changes occurring during the main phenocritical period; the others have already by that time done their work, or at any rate brought it to a point at which it is irreversible. Hence other sensitive periods, much earlier in development, should exist; Goldschmidt[8] suggests that perhaps they occur before each moult.

The phenocritical period has been intensively studied in relation to the formation of pattern, especially in the wings of insects. For a detailed account of this the valuable review of Henke[2, 3] should be consulted, and for short summaries the articles of Strohl[3]. Outstanding studies are those of Kühn & Henke[1] on the wing-colour patterns of the flour-moth *Ephestia kühniella* and those of Köhler & Feldotto on butterfly wing patterns (*Vanessa urticae* and *io*). Goldschmidt ([8], pp. 201 ff.) has tried to assimilate these wing patterns to the pattern of morphological structure produced by the primary organiser of vertebrates, but although differences have been found between areas on the wing preceding the deposition of pigment in these areas (Goldschmidt[3]), we hardly know enough as yet of the mechanism of determination in insect metamorphosis to be dogmatic about this. It is at any rate clear that the pattern is not finally determined at the beginning of pupation, for Henke[1] showed that extirpation of pieces of the wing-forming imaginal discs in the butterfly *Philosamia cynthia* led to a regulative rather than a mosaic development of pattern later. Similarly phenocopies of pattern may be produced by treatment of the pupae of *Ephestia* at the pheno-critical period. Thus the action of genes which shift the two symmetrical bands on the wings centrally, and so suppress the central field, can be imitated by subjecting the pupae from 48 to 60 hr. after pupation (at 18°) to a temperature of 45° for 45 min. (Kühn & Henke[1]), see Fig. 239.

Investigators of wing pattern find that pigmentation seems to start from one point at the base of the wing and spread outwards from there. Or the pattern may be polycentric, as in many ladybird beetles. They therefore speak of a "determination stream" of pattern, which recalls the elongation and shortening of the primitive streak in the chick, and the flow anteriorly and posteriorly of neuralisation from the point of first appearance of the neural folds in amphibia. But the deposition of pigment in a pattern must not be confused with the determination of that pattern. Until further work of an experimental kind has been done on insect wings, the expression "determination stream" might well be used with caution or replaced by "differentiation stream". For further details on this interesting subject the reader must be referred to Goldschmidt's book[8] (esp. the elaborate chapter on pattern). It may be that processes such as those involved in the formation of Liesegang rings will in time be found to occur in the origin of pigmentation patterns, as was first suggested by Gebhardt in 1912.

From what has so far been said it will be clear that external agents such as temperature or spontaneous non-genetic internal agents (as in spontaneous terata) can imitate the action of certain genes. They are therefore to be supposed to affect rates of reaction in the embryo, or to produce hormones, catalysts, or inhibitors which do so. But since 1916 Goldschmidt[1] has applied the same kind

of explanation to genetic dominance. "A dominant gene controls a reacti
a certain velocity; the recessive allele, a reaction of lower velocity, an

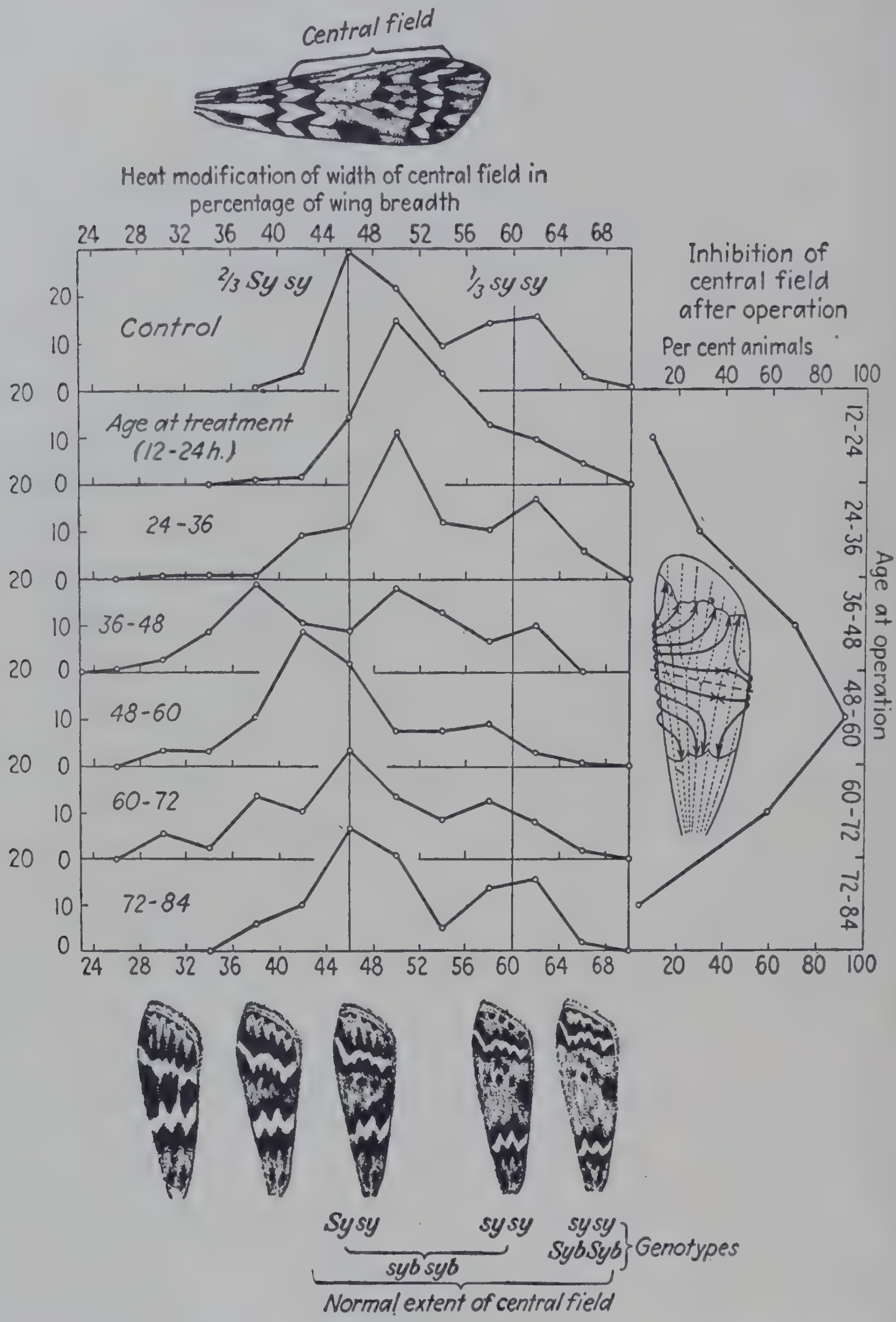

Fig. 239. Suppression of the central pattern-field of *Ephestia* by genic action and by expos
high temperature during the phenocritical period.

heterozygote an intermediate reaction. . . . If dominance is controlled by a dev
mental system, it should be possible to shift the phenotype of the heterozy
by environmental action in the same way as the mutant phenotype ma
secured as a phenocopy." And just as external agencies can shift dominanc

there are certain modifying genes which can do so (dominigenes). But these subjects belong to genetics rather than embryology, and for the details, which are convincing enough, the book of Goldschmidt ([8], pp. 99 ff.) must be consulted.

2·663. Rate genes controlling chemical processes

It only remains to add in this section some further examples of genes which have been shown to affect rates of reaction during development. They mostly concern pigmentation* (cf. the monograph of Stern[1] on multiple allelomorphic series).

Wright[1] and Castle & Wright first showed that a series of this kind exists in guinea-pig coat-colour, where all variations between albinism and maximal melanin deposition may be found. Earlier workers such as Cuénot[1] in 1902 had believed that albino animals contained the chromogen but not the melanin-forming enzyme. The direct tests of H. Onslow[1] in 1915 showed, however, that the enzyme itself was missing from recessive unpigmented areas of skin, while in dominant whiteness a specific inhibitor for the enzyme was present. This was confirmed by Gortner[1] and more recently amplified by Koller[2], with the following general conclusion. In recessive whiteness, mammalian skin cannot make melanin from chromogen, nor can it inhibit melanin formation by other skin extracts. In dominant whiteness, mammalian skin cannot make melanin from chromogen, not because the enzyme is absent, but because it is inhibited; and this inhibitor will prevent melanin formation by other skin extracts.† In recessive blackness melanin formation sensitive to the inhibitor occurs, while in dominant blackness the melanin formation is not inhibitable by the dominant-white inhibitor. Little or nothing seems to be known about the chemical nature of the inhibitor, but after death it may disappear so that the blackening of genetically colourless cells follows, as was found in some old but interesting experiments of Riddle & la Mer on the eyes of embryos of different races of ring-doves. This blackening would not take place if death occurred before the time at which eyes of pigmented races begin to acquire their pigment, suggesting that in the earliest stages the enzyme had not appeared. Post-mortem pigment formation required oxygen, was not inhibited by mercuric chloride, but was stopped by boiling the tissue.

All these facts fit in well enough with the changes in coat-colour which can be produced experimentally in adult mammals such as rabbits by shaving, local subjection to low temperatures, etc. (W. Schultz[2]; Iljin; Iljin & Iljina; Engelsmeier; Danneel; Danneel & Lubnow; Danneel & Paul; Danneel & Schaumann). The new formation of enzyme and chromogen is apparently strictly controlled by skin temperature, which forms an intermediate link in the chain of genetic causation. It seems that the enzyme often appears before the chromogen, e.g. in the cow embryo, according to Esskuchen[1].

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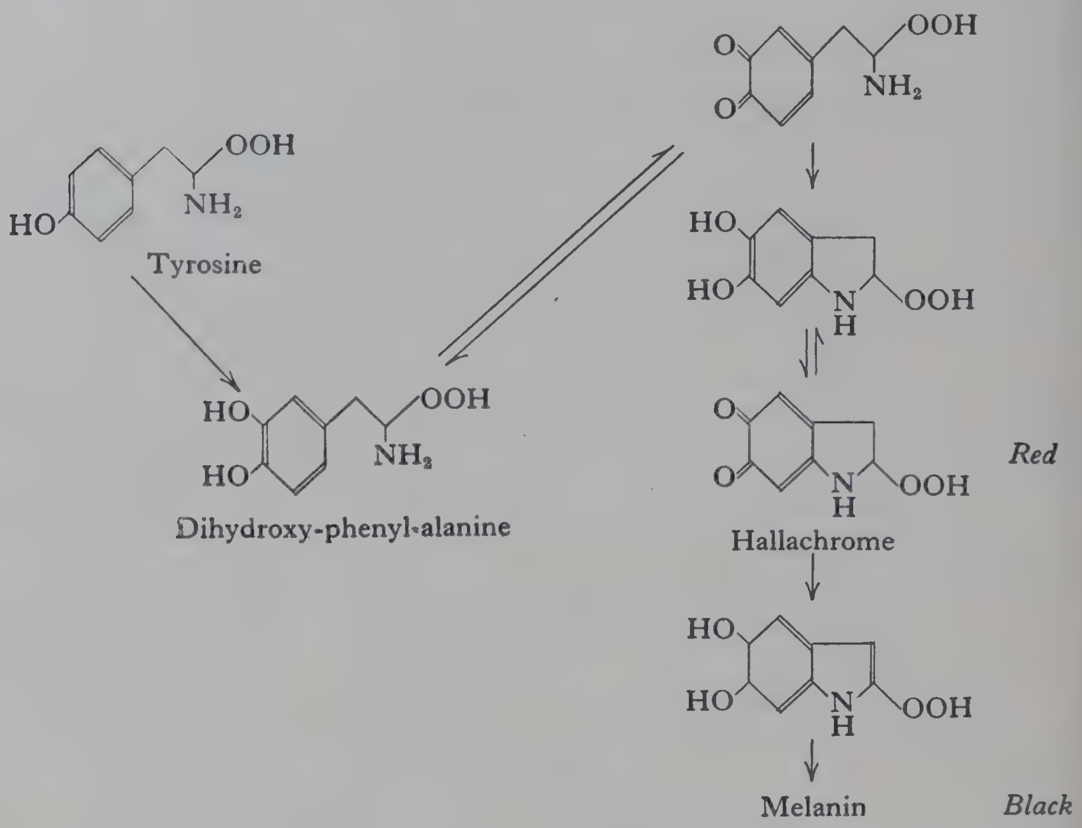
* The most clear-cut results relating genes to pigmentation have of course been obtained in the study of plant flower colours, but these are outside the plan of the present book (cf. reviews by Scott-Moncrieff and Haldane[3]).

† Compare with this the development of melanin in the connective-tissue cells *in vitro*, also studied by Koller[1]. Embryo extract of black Leghorn embryos would not make white Leghorn cells form pigment. Dorris[2] has studied similar problems in transplantations of chick neural crest cells.

The earlier genetical work was carried on in the absence of very clear i
about the chemistry of melanin formation, but when this was to some ext
cleared up (cf. the reviews of Raper—see the formulae below) the way
open for more satisfying work. Einsele's estimation method[2] for melanin m
it possible to investigate the multiple allelomorphic series white-black in
mouse described by Dunn[5]. We know now, therefore, from the work of Dun
Einsele, that the members of this series differ in a truly quantitative way in te
of absolute weight of melanin produced, the difference being expressed morp
logically as a difference of granule size. The absorption spectrum of the mela
of all the members of the series has been shown to be identical by J. Daniel



N.B. Hallachrome, a pigment found by Mazza & Stolfi in the annelid worm *Halla parthen*
is identical with the red intermediate of melanin formation.

The melanin-forming powers of insects have long been recognised to
particularly marked. Insect blood commonly darkens after shedding. Bef
1914 Gortner[2] showed that in the potato beetle *Leptinotarsa decemlineata*
unpigmented elytron contains enzyme (tyrosinase) but no chromogen. Extens
studies were subsequently made on the presence of enzyme and substrate in m
different kinds of insects (Schmalfuss & Werner; Schmalfuss, Barthmeye
Hinsch), but the information gained relates rather to comparative biochemis
and the chemistry of tyrosinase action than to the genetico-embryological probl

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* Work of a similar kind, though relating rather to the numbers of melanophores produced,
been done by Goodrich & Hansen on fishes and by Twitty[6] on urodele amphibia. On tyrosinas
feather germs of bird embryos, see Charles & Rawles.

This was attacked directly in a careful piece of work by Graubard, who investigated various races of *Drosophila*. Wild type, *yellow* and *black* had a high enzyme concentration at all times and stages; in *ebony* it was lower. Inhibitory factors, however, were often, if not always, present; these could be avoided by treating the flies with chloroform before extraction (liberation of chemical substances from a complex?). Graubard came to the probably rather sound conclusion that genetic factors operated not so much on the actual amounts of substrate or enzyme formed, as on complicated internal conditions, such as *p*H, inhibitors, complex formation, etc., the unravelling of which will require much further work.

Eye-colours, as is well known, have afforded much suitable material for genetic studies. The pigment deposited in the eyes of the shrimp *Gammarus* appears to be melanin, and the black stage is preceded by red stages in which the colour is probably due to the red intermediate indol product (see p. 406). Studies of arious races of the shrimp (Ford & Huxley) showed that they constitute a series f multiple allelomorphs characterised by the rate of darkening, i.e. the rate at vhich the whole process from substrate to melanin proceeds. An interesting liagram is here reproduced (Fig. 240). The steepest curve represents the dominant lack-eye type which is black at hatching. Embryos of this type in earlier stages ave pink and then red eyes, though to begin with the eye-rudiment is in all cases olourless. Other genetic types, however, hatch with pink or red eyes and darken nuch more slowly. Emmart has shown that the reactions producing the melanin are more strongly accelerated by rise of temperature than those producing the red pigment.

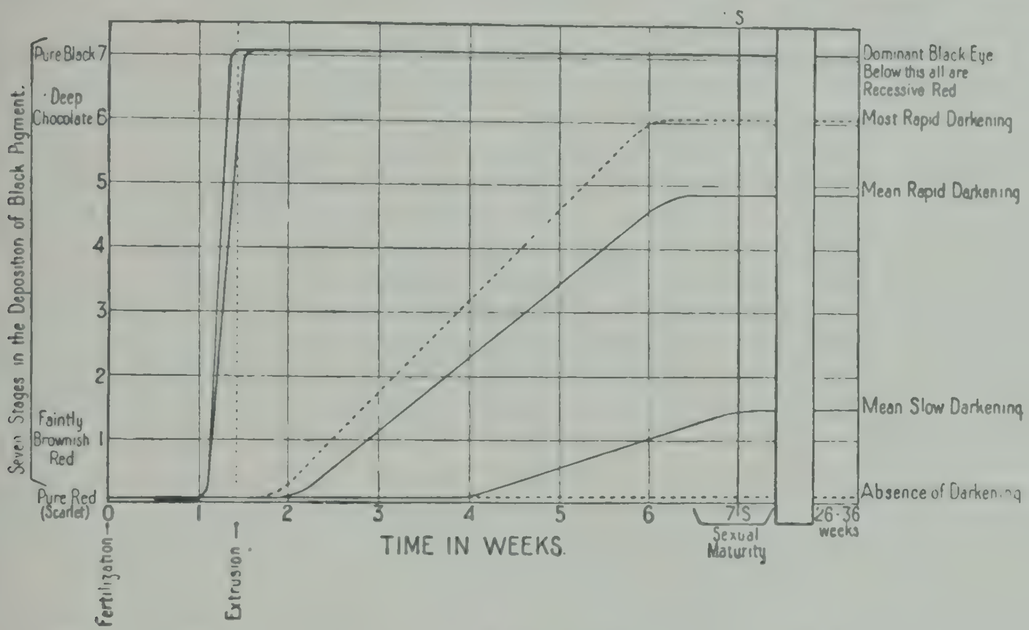

Fig. 240. Changes in eye-colour during the development of *Gammarus*.

By no means all arthropods, however, have melanin deposition as the exclusive pigmentation of their eyes. *Drosophila* has been the subject of important work. lere Johannsen and J. Schultz[2] have found three main pigments, a red, an orange nd a yellow, each contained in a special type of granule. In early pupae the eye s still transparent, but about 75 hr. after pupation the whole eye has become tan in colour. At 80 hr. the tan is rapidly changing to red. An aqueous extract wild-type eyes contains a large amount of a red pigment and smaller amou of a yellow and a brown. The red pigment can be reversibly oxidised to the yell and by treatment of the intact eyes with H_2S the tan colour of the early sta can be immediately changed to red. According to Schultz, all the different types (such as *apricot*, *sepia*, *ruby*, *cinnabar*, *vermilion*, etc.) are due to differ proportions of the three pigments, two of which seem in effect to be the sa pigment in the oxidised and the reduced state.*

By transplantation experiments it has been shown that the colour develop by a *Drospohila* eye may depend not only on its own genetic constitution, on that of the host into which it is transplanted. In other words hormone-l substances produced by genes and governing eye-colours may be at work. T brings us to the end of one section and the beginning of another—nucl inductors.

2·67. Nuclear inductors and nuclear metabolism

We may define nuclear inductors as active substances which are intermedi between the genotypic equipment and the phenotypic array of visible a measurable characters. The case of the *dwarf* mice already mentioned (p. 3 provides a good transition, where the gene differentially suppresses the grow promoting hormone of the pituitary gland without affecting the other hormon But it is now clear that hormones of a non-glandular kind are also involved. animals this has been studied especially in insects (reviews by Ephrussi[6]; Beadl Plagge[6]), but parallel cases have recently been found in plants (review by Ster Melchers; Pirschle).

In 1920 Sturtevant[1] was studying a gene in *Drosophila*; *vermilion*, which produ eyes of lighter colour than those of normal (wild-type) flies. He produc gynandromorphs in which the gonads were always *vermilion* if male, and wild ty if female. When such individuals had mosaic spots† of *vermilion* constitution their eyes, these spots were not *vermilion* if ovaries were present. Hence t simplest explanation was that the wild-type ovary produces a diffusible substan which shifts the eye-colour away from the lightness of *vermilion* to the darkn of the wild type.

Some ten years later, Ephrussi & Beadle[1], who were engaged in success transplants of ovaries between larvae and pupae of different genetic constituti in *Drosophila*, realising the importance of a study of diffusible substances govern eye pigmentation, found that *vermilion* and *cinnabar* eye discs, transplanted in wild-type hosts, developed perfectly and gave wild-type dark pigmentation. Th behaved therefore selfwise from the embryological point of view but neighbou wise from the point of view of genetics (cf. p. 341), since the determinati of the eye was evidently complete, but not of what pigment should be la down in it. After pupation the transplanted eye disc comes to lie free in t abdomen or occasionally in the thorax. There can therefore be no question b that genetically controlled diffusible substances are involved (Ephrussi & Beadle[3]; Beadle & Ephrussi[1]). In a series of experiments in which a large number of all the possible permutations and combinations of eye-disc transplantations were made between some twenty-five different eye-colour mutant races, it was found that under the conditions of the experiments only *vermilion* and *cinnabar* behaved in this neighbourwise, undetermined, way (Beadle & Ephrussi[1]). Reciprocal transplantations therefore acquired great importance, and here the following results were obtained: a *vermilion* eye disc implanted into a *cinnabar* fly gives a wild-type eye, while a *cinnabar* eye disc implanted into a *vermilion* fly gives a *cinnabar* eye.

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* Phenocopies of the lighter eye types have been produced by Rapoport[2] using Sb compoun

† See Glossary.

Ephrussi & Beadle assumed (and all the later results have fitted in with this view) that two different substances are involved, a v^+ substance which converts the pigment from the *vermilion* to the *cinnabar* stage, and a cn^+ substance which converts the *cinnabar* stage to the fully darkened wild-type stage. The body fluid of the wild-type fly contains both these substances; that of *vermilion* contains neither, and that of *cinnabar* contains only the v^+ substance. Since the cn^+ substance always disappears whenever the v^+ substance disappears in a mutant race, although the latter may be present without the former, it is postulated that the two form part of a common reaction chain, the first link of which is the v^+ substance and the second the cn^+ substance:

$$\longrightarrow v^+ \longrightarrow cn^+ \longrightarrow$$

Thus the mutant genes would interfere at different points in this chain. The reciprocal transplantation results just referred to are now explained as follows: a *vermilion* eye disc implanted into a *cinnabar* host gives a wild-type eye because the *vermilion* disc can make cn^+ substance from v^+ substance. That it never does so if left to itself is because it never has any v^+ substance at its disposal. On the other hand, a *cinnabar* eye disc implanted into a *vermilion* host gives a *cinnabar* eye because the host cannot provide either the v^+ substance or the cn^+ substance. The former of these two cases is particularly remarkable because the eye develops wild-type pigmentation in spite of the fact that normally neither the donor nor the host could have produced the cn^+ substance necessary for wild-type pigmentation (Ephrussi & Beadle[3]; Beadle & Ephrussi[1]).*

If these views are correct it follows that we ought to be able to produce the same results on the eye discs by injecting body fluid from flies of different genetic constitution—the converse of the implant method. This was successfully demonstrated when body fluid from wild-type flies was injected into *vermilion* pupae; the eyes subsequently developed wild-type pigmentation (Ephrussi, Clancy & Beadle; Beadle, Clancy & Ephrussi). It was found that only pupae between 3 and 80 hr. from the beginning of pupation yielded active body fluid; larval and imaginal body fluid was almost inactive, so the substance must be formed and then disappear, at any rate from the circulating medium. The point of eye-colour determination was also ascertained. After 70 hr. pupation the eye-colour could no longer be influenced by the injection of body fluid. Later the cn^+ substance

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* Note the logical parallelism here with (*a*) *bedeutungsfremde* self-differentiation (p. 157) and (*b*) the relations of enzymes and substrates in the mechanism of mammalian coat-colour production (p. 405).

was shown to behave in the same way (Ephrussi & Harnly; Harnly & Ephru
with slight differences in the time limits of existence of the active substance,
in the time limits of competence in the responding eye disc.

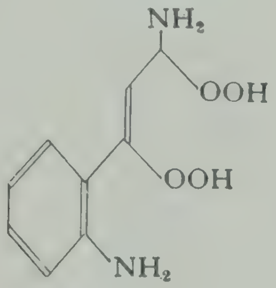
First steps towards the chemical elucidation of the substances were taken
Ephrussi & Harnly, who showed that the action of the body fluid was unimpai
after several treatments with liquid air. The living cells in the fluid had theref
nothing to do with the effect (cf. the early work on the primary organiser descri
on p. 165). Khouvine, Ephrussi & Harnly's extractions of wild-type animals t
showed that ethereal extracts had no activity, but that ether-alcohol, alcohol,
aqueous extracts, were active. This was confirmed by Thimann & Beadle, w
found that very active aqueous extracts could be obtained if the substances w
protected from oxidation by working in nitrogen. The substances were stabl
boiling, and after denaturation of the proteins were also stable to oxidati
indicating that the attack had been enzymatic. No differences between the v^+ a
the cn^+ substances could be found as regards their stability to temperature (b
can be dried at 100° C.) or oxidation by hydrogen peroxide. According
expectation *cinnabar* pupae boiled and extracted proved to contain only t
v^+ substance.

Since Ephrussi & Harnly had shown that the substances were present in t
blowfly *Calliphora erythrocephala* and the waxmoth *Galleria mellonella* (we sh
return to the question of species-specificity; p. 416), Khouvine & Ephrussi work
up large quantities of *Calliphora* pupae. In the ethereal, carbon tetrachlori
or acetone lipin fractions there was nothing active, but the substances could
obtained very active in aqueous medium, and were precipitable with phosph
tungstate and basic lead acetate. No pure nitrogenous bases tried, however, w
at all active. In the most recent paper (Tatum & Beadle[1]), which confirms
the previous work, we find that though the substances are stable to the temperatu
of boiling water for an hour, they are completely destroyed by heating to 160°
for an hour. They are best extracted from dried pupae with 95 % alcohol af
a preliminary extraction with boiling chloroform. They are more sensitive to ac
than to alkali, readily oxidisable enzymically, and soluble in butyl alcohol
*p*H 6·0. They can be precipitated by mercuric acetate and regained with H
By a (somewhat inaccurate) diffusion method, their molecular weight is believ
to be in the neighbourhood of 500. Lastly, according to Khouvine, Ephrussi
Chevais, the substances may be precipitated with silver along with the puri
bases, histidine and arginine, but appear to be destroyed by flavianic, picric a
picrolonic acids, which makes further purification difficult.

Working on the basis of these results, Butenandt, Weidel & Becker found th
the long-known tryptophane derivative, kynurenin, can carry out the action of t
v^+ substance (see formula). Beadle & Tatum find that in its naturally occurri
form this is united with two other molecules, of which one is sucrose.

In the meantime more permutations and combinations of transplantations h
been going on (Ephrussi & Beadle[3]; Beadle & Ephrussi[2, 3]), particularly with rega
to the case of *claret*, which is rather hard to interpret. It was shown that t
structure of the transplanted eyes develops normally, so that if there is a

"inductive" action of the optic ganglion upon the eye, it must occur before the usual time of implantation (Chevais[1]). But the most important line of work was perhaps that of Beadle[1], who identified the fat bodies and the Malpighian tubules as the normal sources or depôts of the substances. Wild-type fat bodies have the v^+ substance but not the cn^+ substance; the Malpighian tubes have both. From larval fat bodies nothing can be extracted, nor from salivary glands, brain, or hind-gut. In the Malpighian tubules the substances are present as soon as 24 hr. after hatching, and larval Malpighian tubules lacking them can take them up if they are injected into the body-cavity. Beadle believes that the Malpighian tubes are actually the site of synthesis of the substances.*



Kynurenin

Next it was found by Beadle & Law that the substances can enter the body of the developing larva by the mouth and still exert their action. *Vermilion* and *cinnabar* larvae fed on cooked wild-type pupae 60–85 hr. after the beginning of embryonic development will later have wild-type eye-colour. This led to an interesting investigation of phenotypic effects exerted by the culture medium (Khouvine, Ephrussi & Chevais). Feeding *Calliphora* extract in agar had the same result, but it was found that starvation also brought about modifications of eye-colour of *vermilion* flies in the wild-type direction. Small doses of dry yeast had this effect, but larger doses suppressed it. Small amounts of peptone gave the same result, and this was abolished by larger amounts. The large doses of yeast extract suppressed the peptone effect but not the effect of feeding *Calliphora* extract. Gelatin peptone was inactive, but became active on the addition of tryptophane. Not one of these effects concerned *cinnabar* flies. Yeast extracts injected into the body-cavity had no action on eye-colour.

These curious phenomena are provisionally explained as follows: normally *vermilion* flies have all the metabolic machinery for making v^+ substance, but at some point or other it is diverted. Starvation restores the mechanism to its normal state. As for the feeding experiments, yeast and peptone must contain some precursor substances which in the protein enzyme systems of the gut are transformed into the active eye-colour inductors. In living yeast (on which *vermilion* flies can be raised) the substance must be either absent or masked.

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* It is now known that some bacteria can synthesise the v^+ substance or something remarkably like it (Tatum). Cf. the bacterial synthesis of vitamin K (Almquist, Pentler & Mecchi), and the synthesis of vitamin B_1 in refection.

An unmasking mechanism might also account for the fact that if *vermilion* larvae
fed on cooked *vermilion* pupae a shifting of eye-colour towards wild type occu
The starvation problem was also studied by Beadle, Tatum & Clancy, who fou
that before 70 hr. from egg-laying starvation would retard larval growth, but
afterwards. The sensitive period for the starvation effect on eye-colour occ
just before this change (Beadle, Tatum & Clancy; Tatum & Beadle[2]; Rudki

In what has so far been said, the question of the substrate on which the e
colour inductors act has been omitted. But eye discs of *vermilion* or *cinna*

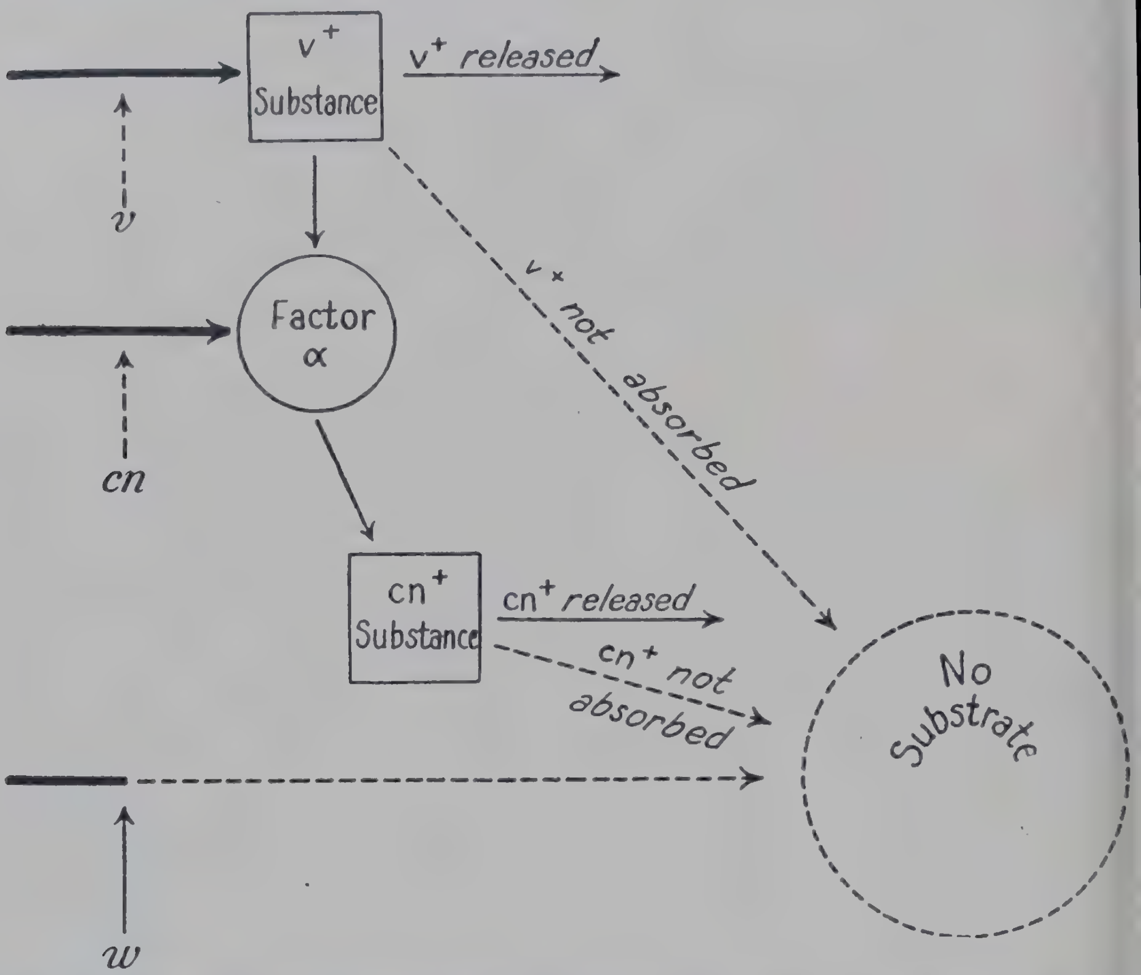

Fig. 241. Scheme of the relations between diffusible substances and eye-pigmentation. (A) c
of eyes with no substrate.

pupae transplanted into larvae of the mutant *white* develop into eyes which
wild type in colour. Ephrussi & Chevais concluded therefore that the *white* pu
must possess the two necessary substances, and that the total absence of pigme
in its eyes must be due to the disturbance of some mechanism other than th
which leads to the formation of the diffusible substances. As Beadle & Ephru
had previously shown that eyes may themselves give off one or other of t
substances, a series of eyes of *white* allelomorphs (*honey*, *cherry*, etc.) w
implanted into suitable hosts, and it was found that the release of the v^+ substa
was inversely proportional to the intensity of pigmentation of the eyes used. T
same proportionality was found for the cn^+ substance. This suggests that t

whiteness of the eye may be regarded as a measure of the eye's inability to utilise the substances presented to it.

Ephrussi & Chevais summed up all their results in the two diagrams here reproduced (Figs. 241 and 242). The *cn*+ substance is thought to result from the interaction of the v^+ substance with an intermediate product called factor α. Formation of the v^+ substance and that of factor α can be interrupted independently of each other by the genes *vermilion* and *cinnabar*. But the substrate or chromogen in the eye is also variable; its appearance is inhibited to a greater or lesser extent by the *white* allelomorph gene series. In the wild-type eye the v^+ and cn^+ substances act upon this substrate. But when the substrate is absent, these substances would hardly be utilised at all by the eye and would therefore thus released be capable of influencing another eye (Fig. 241). When the eye is rich in substrate, the inductors would be utilised, fixed, or consumed, and therefore not released. Intermediate cases of all grades between these two extremes can be imagined.

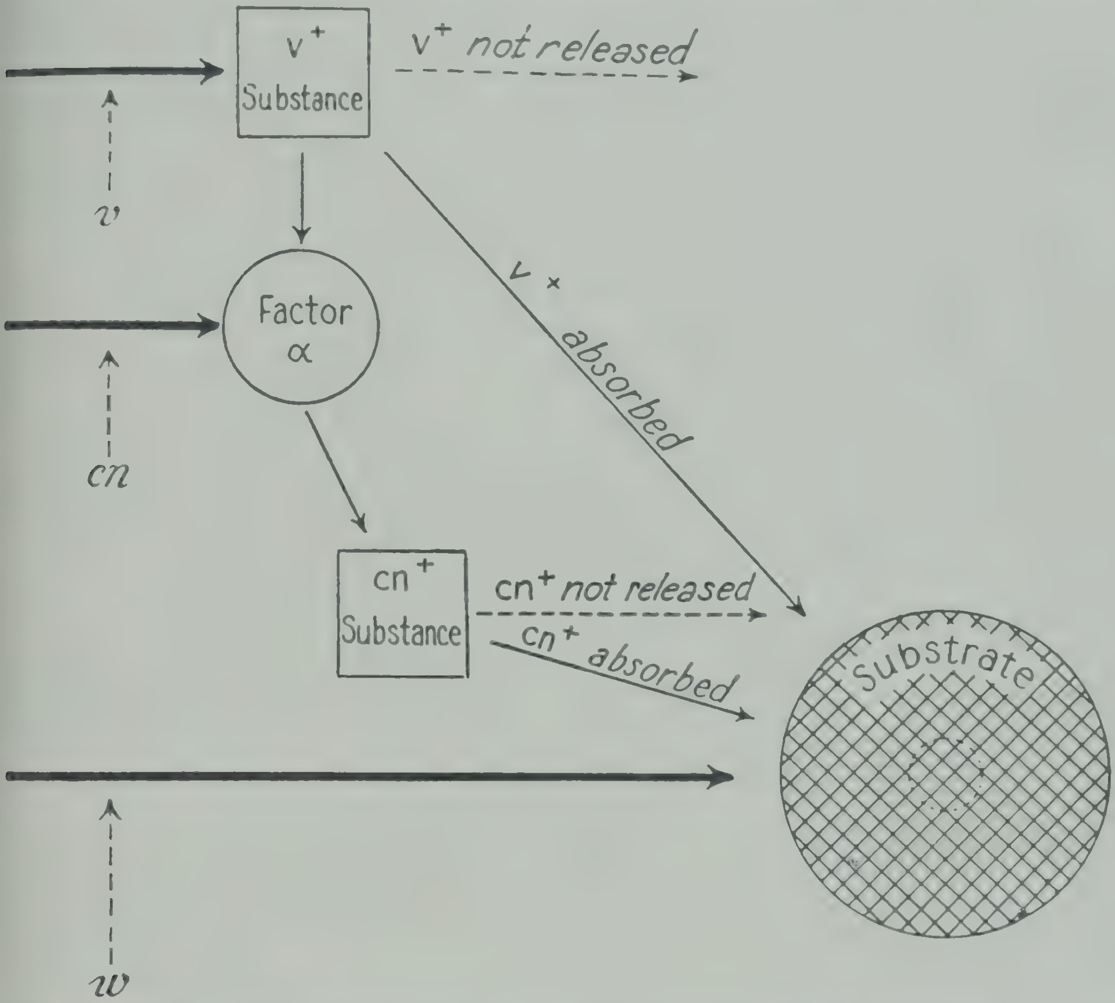

Fig. 242. Scheme of the relations between diffusible substances and eye-pigmentation. (B) case of eyes rich in substrate.

In sum, then, it appears probable that all the variations of these eye-colours in *Drosophila* are due to the production of varying amounts of a few water-soluble diffusible "nuclear inductors", possibly three in number, certainly not less two.*

In reviewing the great extension of our knowledge of nuclear inductor described, the most seriously backward side of the problem seems to be an knowledge of the eye pigments themselves and the reactions which lay down. The reader must often have been reminded of the progressive dark to black through red described in the eyes of *Gammarus* by Ford & H (see Fig. 240). There the red pigment may well be hallachrome and the blac melanin. The *Drosophila* eye pigment picture seems to resemble this suspiciously, but with the added complications of some lipochromes (cf. the of Schultz, p. 408); if this resemblance is an illusion we urgently need to the facts more clearly. Chevais[2], indeed, has shown that the effect of th substance is to accelerate the time of appearance of pigment in *vermilion* by as much as 20 hr. from the beginning of pupation. This fits in well wit work of K. Mori but especially of Cochrane[1] on the course of pigment develop

Cochrane distinguishes between early-phase pigment granules, which ar down from the 96th hour of pupal life onwards, and late-phase granules, are not laid down till after the 120th hour. The early-phase granules change yellow to brown in the primary pigment cells and from yellow through b to red in the secondary pigment cells. The late-phase granules appear on the secondary pigment cells and change from yellow through orange to According to Cochrane, genes act either by suppressing granule formatio by altering the rate of colour change, or by qualitatively changing the tints. *vermilion* suppresses all the early-phase granules, though the late-phase gra appear and develop just as in wild-type flies. *White* suppresses all gra completely. *Sepia* stops the reddening of the late-phase granules. The a morphic series of *purple* works as follows: *purple*[2] retards the change from y to red of the late-phase granules; *purple*[3] slows the reddening of both early- and late-phase granules; and *purple*[1] while slowing the reddening of the e phase granules suppresses the late-phase granules altogether. These result illustrated in the accompanying diagram (Fig. 243). Literature relating t histology and embryology of insect eyes is given by Cochrane in her paper

Attempts have been made to find cases of genetically neighbourwise o determined development in other directions by transplantation between ge races (e.g. body colour by Medvedev[2], and bristle formation by Glancy & land) but so far without success.†

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* The line of distinction here between hormones in the usual sense and precursor-substa perhaps thin. It has not yet been shown that the nuclear inductors for eye-colours are q tatively minimal in relation to the pigments produced by their action, as is the case with hor They may, therefore, resemble melanin precursors. But all hormones may be precurso stances; the resultant molecules may include them as constituent parts of which very li needed.

† A case was formerly thought to exist in birds. Willier, Rawles & Hadorn transplanted ect between chick embryos of white and coloured breeds; it was found that while the presu coloured feathers never became white, presumptive white ones would develop neighbour far as pigmentation was concerned. Rawles[2] found, however, that this was due to the mig of melanophores. Exactly the same explanation holds good for testis coloration in *Dros*

As regards species-specificity, Howland, Glancy & Sonnenblick first found no differences between the various species of *Drosophila* in production of v^+ substance. Then, as has already been mentioned, Ephrussi & Harnly found the v^+ and cn^- substances present in extracts of blowfly and waxmoth tissues (cf. the lack of species-specificity of the primary organiser of vertebrates). Though the question of the identity of various nuclear inductor hormones in different insects is not yet quite settled, it appears that the species-specificity is very low (Beadle, Anderson & Maxwell). Similar diffusible substances have been reported for the

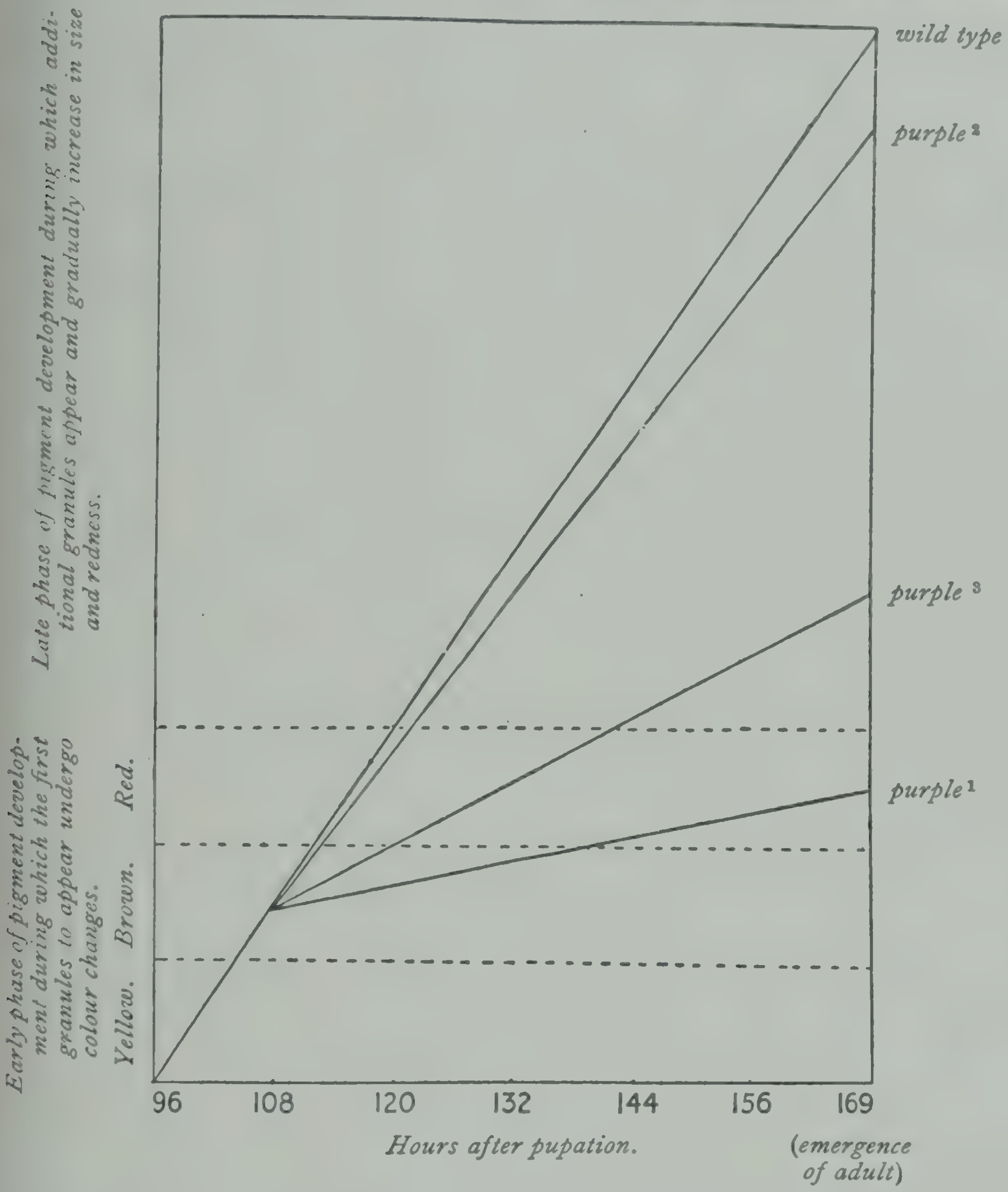

Fig. 243. Action of genes on eye-colour in *Drosophila pseudobscura*.

(Stern & Hadorn). But if we include shape as well as pigmentation there is the case of testis form n *Drosophila* reported by Stern[2]. The "genetically spiral" testis of *D. azteca* assumes an ellipsoidal shape if attached to the vas efferens of *D. pseudobscura*, and *vice versa*. This is in striking contrast to the amphibian results described in Section 2·61 where the genetic constitution of the reactor determines the nature of the response to the inductor. It is so far the only case of its kind.

silkworm *Bombyx* (Kikkawa[1]) and for the parasitic wasp *Habrobracon* (Whitin Whiting & Whiting). Proof of the identity of such substances was given by Beck & Plagge[1]. In the course of work on the red-eyed mutant race of the mealmo *Ephestia kühniella*, which will now be described, it had been found that extra from the black-eyed wild type (*AA*) would darken the eyes of the red-eyed ra (*aa*). When such extracts were tested on *vermilion Drosophila* the result was ju as if wild-type *Drosophila* extracts or transplants had been used. Converse *aa Ephestia* extracts produced no effect on *cinnabar Drosophila* pupae (Plagge Becker). This shows that the change from *A* to *a* in *Ephestia* corresponds to t change from wild type to *vermilion* in *Drosophila*, because *Ephestia aa* contai nothing from which *Drosophila cinnabar* can make the cn^+ substance. So bo the substances and the reaction chain seem to be the same in the two insects.

The analysis of the *A*-hormone effect in *Ephestia* had begun before that of t eye-colour substances in *Drosophila*. In 1930 Kühn & Henke[2] had described "pleiotropic" gene which affected the pigmentation of many different parts the moth. Thus in the wild-type moth the imaginal eyes are black, the testes ar the brain brown, the larval eyes dark and the larval skin red. In the red-eye mutant (*aa*) the imaginal eyes are red, the testes colourless and the brain almo so, the larval eyes pale and the larval skin white and transparent.* There i therefore, a general inhibition of pigmentation. Three years later Caspari foun that when a wild-type testis is transplanted into an *aa* larva, the *aa* testis darke towards the wild-type colour. A diffusible substance is therefore present in which can reverse the effect of the *aa* gene. The same holds true for many oth wild-type organs and for other reactive tissues such as the eye (Kühn, Caspa & Plagge), and the substance missing from *aa* larvae is called the *A*-hormone.

A-hormone is produced by ovary, testis and brain, and like the v^+ and *cn* substances of *Drosophila* it can diffuse out of eyes. It appears in the body flu of wild-type moths during the larval stage. Testis from very young wild-typ larvae implanted for a 2-day period into 9-day-old *aa* pupae and then remove exert a considerable darkening effect upon the eyes of the host (Plagge[1]). Plagg found that a stay of 24 hr. in the body of the *aa* host is necessary for the effe to take place in some individuals; lesser periods of time do not allow of the diffusio of sufficient *A*-hormone, and by 72 hr. nearly all the hosts show the effect.

One remarkable effect which does not appear in *Drosophila* was seen i *Ephestia*, namely the use of the *A*-hormone in the succeeding generation (Küh Caspari & Plagge; Kühn & Plagge; Plagge[4]). If a heterozygous *Ephestia* ♀ back-crossed to an *aa* ♂, it is found that the homozygous *aa* offspring have colours as larvae and *aa* colours in the adult state. This must be due to the stora of the *A*-hormone of the heterozygous mother in the eggs, because if testes fro wild-type moths are implanted into ♀♀ larvae of the red-eyed race, and if t resulting moths are crossed to red-eyed ♂♂, the offspring again show *A* larv characters and *aa* adult ones. The small amounts of *A*-hormone contained in heterozygous moth have thus here been replaced by *A*-hormone from test transplantations into homozygous *aa* ♀♀. The hormone may thus exert its effe by "maternal inheritance" (see p. 366), being stored in the eggs and utilised later on.

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* Cochrane[2] finds some association between eye and testis colour in *Drosophila* also.

Periods of competence have been identified in *Ephestia* as in *Drosophila*. On the 9th day after the beginning of pupation the eye-colour is quite undetermined and can be influenced by wild-type testis implantation; but from the 12th day onwards this effect can no longer be obtained (Plagge[1]). This point is earlier relatively than in *Drosophila*. Competence for change in testis pigmentation is already over before the beginning of pupation. Competence is lost from different parts of the eye at slightly different times.

Just as in *Drosophila* two substances are involved, so in *Ephestia* it seems that the *A*-hormone of the gonads is slightly different from that of the brain (Kühn). Implantations of gonads affect eye and gonad pigmentation with equal intensity; implantations of brain affect eye pigmentation without changing gonad pigmentation very much. The two substances must, however, be very closely related. They occur (cf. the lack of species-specificity in the case of *Drosophila*) in many other species, such as *Acidalia virgularia*, *Laspeyresia pomonella*, *Plodia interpunctella* and *Plusia chrysitis* (Plagge[3]). They have been chemically investigated by Becker[1], who found, as in the case of the Beadle-Ephrussi substances, a ready enzymatic oxidation, which could be overcome by making acetone extracts of *A* race abdomina. He concluded that the *A*-hormone cannot be of protein or lipoidal nature, which, so far as it goes, corresponds with what is known of the Beadle-Ephrussi substances.

The drawback about all the preceding researches, valuable though they are, is that the end-results of the nuclear inductors are comparatively trivial, consisting as they do of pigmentation changes. The demonstration of nuclear control over morphogenesis requires end-results of a more clearly morphological character. There exists, it is true, one line of investigation which provides what we are looking for, but unfortunately only for one of the lower plants—the work of Hämmerling on the umbrella alga, *Acetabularia*.

This alga has the form of an umbrella, in the "handle" of which, i.e. at the root or rhizoid end, the single large nucleus is situated. Although the alga is but a single cell it is a giant one, reaching 2 or 3 cm. in height; and the diameter of the umbrella may be as much as 0·6 cm. When the stem is cut at any place, it is found that both the nucleated rhizoid at the "posterior" end and the anucleate umbrella at the "anterior" end are capable of regeneration. The anucleate umbrella end will live for a considerable time before dying, the nucleated rhizoid will of course continue to live indefinitely. Hämmerling[1] found that the regeneration of the umbrella takes place progressively more readily towards the anterior end, and that regeneration of the rhizoid takes place progressively more readily towards the posterior end. The plant is thus polarised along its longitudinal axis. But the amount of regeneration possible for any piece of the plant in isolation from the nucleus is strictly limited, and Hämmerling[4] was able to summarise all his findings in the hypothesis that the nucleus produces two "morphogenetic substances" which diffuse so as to take up positions in a gradient system, there being progressively more of the umbrella-forming substance towards the anterior end and of the rhizoid-forming substance towards the posterior end. During regeneration these substances are used up, and if no nucleus is present, they cannot be replenished. The substances must be rather stable, as a pe regeneration of an umbrella may occur if the umbrella is cut off from its s two months after removal of the nucleus from the root. On the other hand, nucleated plant is cut at a point fairly near the posterior end, an umbrella be regenerated at the cut surface although no umbrella would normally have h formed in that region; if now the nucleus be removed, regeneration will conti until the morphogenetic umbrella-forming substance has been used up, a which it will stop, never to be renewed, as the source of the substance has b taken away.

That the nucleus was also the source of genetic characters was proved Hämmerling[2] using two different species, *Acetabularia mediterranea* and *w* *steinii*. Anucleate fragments could be grafted on to nucleate fragments in b senses. The nucleus then always controlled the differentiation, so that, example, a *mediterranea* stem upon a *wettsteinii* root forms a *wettste* umbrella.*

The brilliant work of Hämmerling differs from other studies on gradients plants and animals of stem-like organisation (discussed by him in a sepa paper; Hämmerling[3]) because of the decisive advantage possessed by the prese or absence of only one nucleus. But it has also its demerits, more particula the fact that the morphogenetic substances or nuclear inductors remain hy thetical, and that no chemical work has so far been carried out upon them.†

From all that has been said in the preceding sections, it will have become cl that the genes act in development by producing, inhibiting the production or masking and unmasking, hormones, catalysts or inhibitors in more or diffusible states. Hence their undoubted effects upon rates of reaction in embryo. *It might even be said that the main difference between genetics and* *bryology is that in the former study, only those inductors are described which unable to pass out through the cell-membranes of the cells in which they are form while in the latter, only inductors of a more diffusible nature are described.*‡ this would be to leave out the whole question of competence, which is likely remain mysterious long after our present knowledge of genes and inductors quadrupled its present state.

There is, however, one approach to nuclear phenomena in embryology wh has not yet been mentioned, namely the direct examination of the egg nucleus the micro-methods which are now becoming available. This kind of work is o just beginning. The early work on oxidation-reduction potential by injection of indicators failed to give any evidence of differences from the cytoplasm (*CE*, p. 846). More important was the work on the metabolic rate of non-nucleate and nucleate fragments of echinoderm eggs obtained by ultra-centrifuging (Shapiro[4]; Navez & Harvey); this will be discussed in detail in the section on embryonic respiration (p. 573), but it clearly showed that there was no particularly strong respiration of the nucleus, and that a non-nucleate fragment could have a much more rapid oxygen uptake than that of the whole egg. The question was examined directly by J. Brachet[15], who measured the CO_2-production (micro-titrimetrically) and the O_2-consumption (Gerard-Hartline method) of the isolated nuclei of *Rana fusca* oocytes nearly at the end of their growth. The metabolic rate of these germinal vesicles turned out to be, in spite of their large size relative to the size of the oocyte, only 1·5 % of the metabolic rate of the latter. Added glucose or cytoplasm gave no increase in the metabolic rate of the nucleus. Later J. Brachet[20] compared the dipeptidase activity (alanylglycine) and the esterase activity (methyl butyrate) of the nucleus and cytoplasm of the amphibian oocyte, using the micro-methods of the Carlsberg school. No difference whatever was detectable between the dipeptidase activity of nucleus and cytoplasm, volume for volume (about $1{\cdot}2 \times 10^{-3}$ mgm. amino-N/hr.), but the esterase seemed to be more active in the cytoplasm than in the nucleus (in c.mm. $N/20$ HCl/20 hr./37°; 0·15 for the nucleus and 0·43 for the cytoplasm.

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* As Holtfreter points out ([13], p. 417) these results are also in contrast with amphibian xenopla transplantations, where the graft, though reacting to the inductor, does so in its own genetic characteristic way (cf. pp. 341 ff.). But there the graft brings its genetic equipment with it.

† In connection with the production of morphogenetic substances by the nucleus, the experim on the relative power of the amphibian nucleus and cytoplasm in neural induction, mentio on p. 171, should be recalled. Some cytological evidence also exists pointing to a dispersio nuclear agents from the "lampbrush chromosomes" of the amphibian oocyte (Waddingto p. 101).

‡ Cf. p 140 where intermediate cases are described, in connection with mosaic development, p. 312 where the inductors of sex-differentiation are described. The same thought recurs p. 289 in the discussion of Huxley's classification of hormones[13], and on p. 669 where in Whitak plant eggs[19] the furthest distant mutual effects are seen. Obviously none of these borderlines be sharply defined. Medawar's phrase[2] has summarised the matter; "a large part of the phy of developmental mechanics is primarily a physics of diffusion."

Similar work on echinoderm eggs, carried out by Holter & Linderstrøm-Lang, gave the same result. No perceptible effect of the nucleus could be found in the centrifuged nucleate and non-nucleate portions of eggs of *Dendraster excentricus*. Against the criticism that the germinal vesicle had already dispersed its contents into the cytoplasm, similar experiments on amoebae, in which the nuclei were removed with a micro-manipulator (Holter[2]; Holter & Kopać), gave exactly the same result. It remains of course to be seen whether other enzymes will also be found to have no connection with the nucleus.

In one respect, however, the nucleus shows a striking difference from the ytoplasm. As has already been described (p. 203), J. Brachet[18] has shown how he nucleus of the mature amphibian oocyte gives an intense nitroprusside test fter fixation with trichloracetate, while the cytoplasm gives practically none at ll. Still more significant, the zone of this reactivity leaves the nucleus during he cleavage stages and ascends to the animal pole of the egg, coming to occupy ust before gastrulation precisely that portion of the gastrula which is to invaginate nd to possess and exert the inductive power of the primary organiser. As yet ve have no idea of the meaning of this distribution of the fixed –SH groups. But it is interesting to note that a large amount of work has given rise to the belief, whether well or ill founded, that the sulphydryl group has something to do with cell-division and even with the control of embryo and adult size. At this point, therefore, it will be worth while to examine this literature, and in so far is the sulphydryl group is believed to be the intermediary between genes and henotype, it may be regarded as a nuclear inductor elect. What its claims are o assume this office we may see in the following section.

2·68. The control of embryo and adult size

We must first discuss the supposed relation of the sulphydryl group to
division. One of the earliest observations made by Hopkins[1] in the discover
glutathione was that the blastoderm of the chick gives a positive nitroprus
test (in the laid but not incubated egg) while the yolk and white do not. For s
time past it has been known that the glutathione content of the embryo falls
age, just as does growth rate, mitotic rate, etc. (evidence in *CE*, Section 1

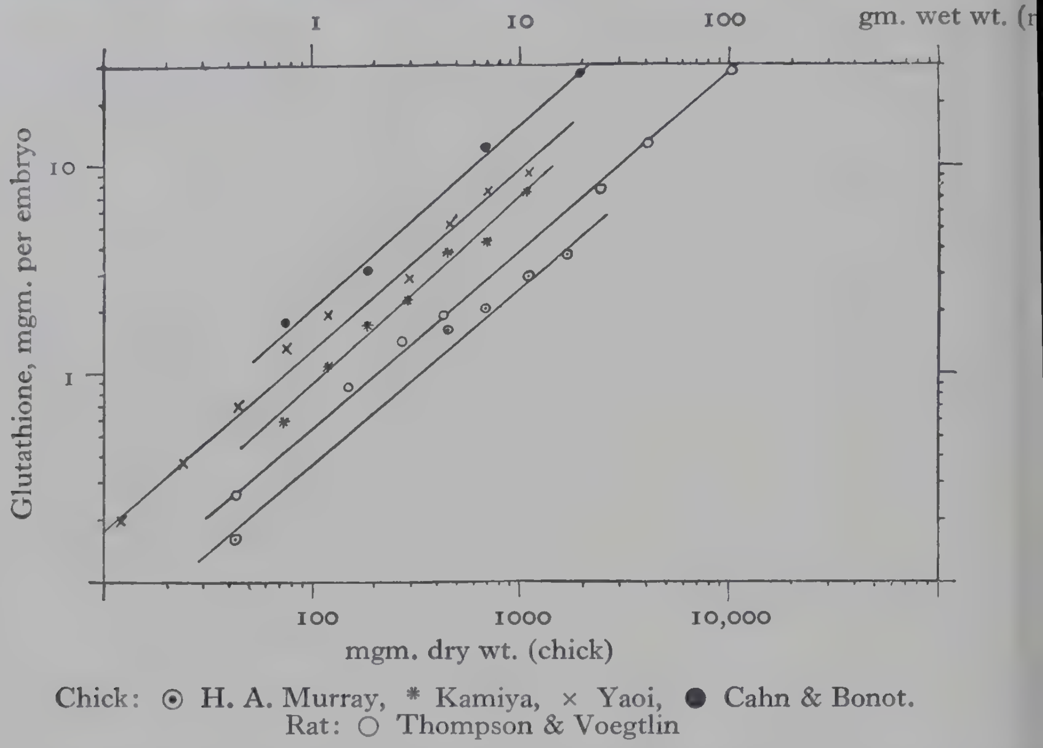

Fig. 244. Glutathione in the embryo of chick and rat (various investigators) plotted on a do
logarithmic grid. The decline in glutathione with age (negative heterauxesis) is shown by
fact that the sets of data are all inclined at an angle of less than 45° to the abscissa (see Section 3·
It may be noted that the heterauxetic constant is very similar in all cases, even that of the ra

		k
Chick	Murray	0·84
	Kamiya	0·90
	Yaoi	0·87
	Cahn & Bonot ...	0·88
Rat	Thompson & Voegtlin	0·85

The decline with age is also seen in the figures of Bierich & Rosenbohm for rat brain and poss
liver, but they are not suitable for plotting as insufficient weight data are given.

For example, the glutathione of the chick embryo falls from 400 mgm. % dry
at the 5th day of incubation to 100 mgm. % dry wt. at the 20th, and that of
rat embryo from 60 mgm. % wet wt. at 0·07 gm. to 36 mgm. % wet wt. at bi
These relationships have been independently confirmed by Kamiya and Tateis
Fig. 244 illustrates the fall of –SH with age in the chick and rat foetus, in
form of a double logarithmic graph, reduced glutathione being brady-aux
(see pp. 533 ff.).

Thus the younger the tissue the more glutathione it contains (relatively).
high glutathione content were always associated with cellular proliferatio
might be expected that tumour tissue would show high values, but though th

has been considerable dispute about this (literature in the reviews of Voegtlin and B. E. Holmes[3]) the upshot seems to be that the glutathione content of mammalian tumours is neither unusually high nor unusually low.

As regards the echinoderm egg, Shearer stated already in 1922 that there was a considerable increase in reducing –SH on fertilisation, as judged by the nitroprusside reaction. This result was accepted with some reserve at the time, but has since been confirmed rather strikingly by the histochemical work of Dulzetto, who found a similar increase in the intensity of the nitroprusside reaction at fertilisation. No perceptible change occurred in its intensity up to the time of the first cleavage. However, histochemical methods could not have the final word in deciding the existence of such a change, and Rapkine[3] measured quantitatively the glutathione (free –SH) content of echinoderm eggs during their early development. He found that the rise at fertilisation is to a level of 40 mgm. –SH/100 gm. wet wt. (from about 30 mgm.). It is followed by a fall to a minimum of 10 mgm. % at 30 min. from fertilisation, and then by a rise to a level of 45 mgm. % just before the first cleavage. This may be regarded as fairly good evidence for his view that segmentation is preceded by increase of sulphydryl groups. Additional evidence is afforded by histochemical nitroprusside reactions tried on ciliates at different stages of division by Chatton, Lvov & Rapkine and by the work of Ephrussi on explanted cells. Ephrussi[1] found that tissue cultures in non-nutritive media gave positive nitroprusside reactions so long as their growth was continuing, but ceased to do so as soon as it stopped. Even if heated to 100° these exhausted cultures gave no nitroprusside reaction, but those still in growth gave an enhanced one owing to the denaturation of the proteins.

Quite independently, certain American workers had been led to envisage a connection of some sort between sulphur metabolism and cell-division. Hammett[1] found that the length of the roots of *Allium cepa* and *Zea mais* growing in very dilute lead nitrate solutions was inversely proportional to the concentration of lead. He also believed he had found a diminution in the number of mitoses, and no effect on cell size. If inhibited roots were transferred to solutions of substances containing the –SH group, there was found an augmentation of length as against the controls. In Hammett's view, these phenomena were to be explained by a combination of the lead with the sulphydryl group and recommencement of mitosis if additional –SH was provided. Normal growth, moreover, was found to be stimulated by the sulphydryl group, and in work with *Paramoecium* cultures, much the same state of affairs was found. In a long series of papers Hammett and his collaborators* extended this theory to wound healing, skin proliferation, crustacean limb regeneration, etc. In later papers of the series, Hammett[3] extended his views to cover the oxidation products of sulphur and claimed that sulphur compounds such as sodium *p*-toluene sulphinate, di-*p*-toluene sulphoxide, di-phenyl-sulphone, etc., exert an inhibiting effect upon growth and mitosis.

Hammett's views were quickly tested by a number of other workers but not with confirmatory results. No effect of sulphydryl compounds on the rate of development of the eggs of the snails *Physa* and *Limnaea* could be detecte Gaunt, who threw doubt on the validity of Hammett's use of amino-acid solu as controls. T. P. Sun[1], again, was unable to accelerate the rate of cell-div in the sea-urchin egg by treatment with H_2S. Morgulis & Green, who triec effect of sulphydryl groups on regeneration in the polychaete worm, *Pod* found no evidence of any cumulative advance in the regeneration of the ex mental animals as compared with the controls. Morgulis & Green brought se criticisms against Hammett's methods of calculation and expression of res and particularly against the use of onion-root tips in mitotic counts (cf. criticisms of Bateman[2]; Moissejeva[1,2]; Richards & Taylor; Taylor & Ha Rossmann; von Guttenberg; Gray & Oeillet; Hollander & Claus; etc. ag the work on "mitogenetic rays").

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* Hammett[2]; Hammett, Anderson & Justice; Chapman; Hammett, Green & Davenport; Hammett & Hammett; Hammett & Justice; Hammett & Reimann; Reimann; Hammett & Smith; Hammett & Wallace; review by Hammett[3,4]; critical review by Hueper.

Not all the investigations were negative, however. Sulphydryl was foun stimulate growth (cell-division?) in planarian worms (Coldwater[1]), and in ti cultures (Baker[1]). In connection with the latter, it was found by Sachs, Rap & Ephrussi that a large part of the reducing power of normal embryo ext as prepared for tissue cultures, is due to sulphydryl groups reversibly block by iodoacetate. But the most convincing results were those of Voegtli Chalkley[1], who found a marked acceleration of nuclear and cell-division in amo subjected to low concentrations of glutathione both in the reduced and oxid form. This work, in which the differences between the control cultures and t receiving glutathione were sometimes very substantial, and which had as a b ground Chalkley's careful observations[1] of normal maturation and cell-divi in amoebae, seems rather reliable. Chalkley & Voegtlin also found that co in minute concentrations exerts an inhibiting action on cell- and nuclear-divis and that there is an antagonism between copper and glutathione. Later it shown that mitosis in amoebae is reversibly inhibited by H_2S and HCN but by CO even in darkness (Voegtlin & Chalkley[2]).

On the whole, however, it cannot yet be said that the causative action of ra –SH concentration on cell-division has been definitely proved. But this not destroy the basis of Rapkine's belief in a connection between sulphy groups and cell-division, which rests on other grounds as well.

The original experiments of Hammett on lead were continued with merc Hoadley[4] showed in an interesting paper that very regular inhibitory effects c be obtained by treating sea-urchin eggs with very dilute solutions of merc chloride. After fertilisation, he exposed them for short periods to the actio the heavy metal, and then returned them to normal sea-water. As Fig. 245 (dr from his data) shows, the relation between exposure time and time required the first egg of the batch to cleave for the first time is practically linear. higher concentrations of mercury in the protoplasm (longer exposure time) development is more affected, since the curve relating exposure time to percen of swimming embryos after 18 hours drops rather suddenly after a certain p is reached. The work was continued by Rapkine[3], who found similarly that mercuric chloride echinoderm development can be stopped at any given s in 100 % of the eggs, but also that it is completely reversible if they are transfe to sea-water containing cystein or thioglycollic acid. Controls transferre sea-water alone or to sea-water plus alanine or cystine manifested a recovery ranging from 0 to 80 % of that in sea-water plus –SH, with an average of 34 %. So far the experiments of Rapkine were analogous to those of Hammett and Hoadley on other material, but he went further in showing that from echinoderm eggs the thermostable residue of Hopkins & Dixon, i.e. a protein preparation containing fixed –SH, could be made. This lost its reactivity to nitroprusside immediately if treated with mercuric chloride. And mercuric chloride would also inhibit the reduction of methylene blue by thermostable residue and cystine in the Thunberg tube.

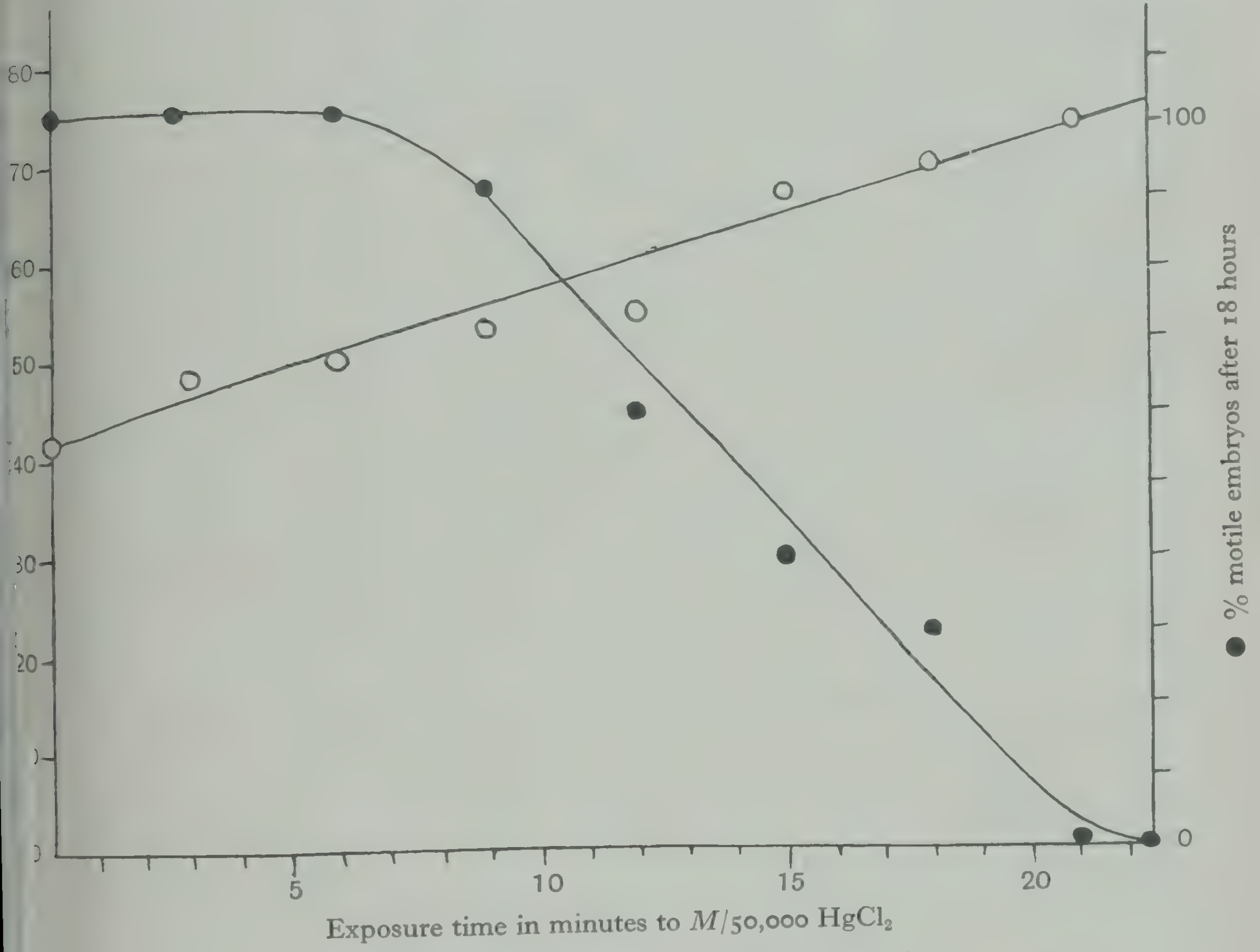

Fig. 245. Effect of Hg on *Arbacia* eggs (Hoadley).

One more link in the chain of reasoning remains. Rapkine supposed that the essential phenomenon causing the increase of –SH before cell-division was the increase of –SH groups attached to protein molecules, i.e. fixed –SH. We know that the denaturation of a protein is often accompanied by the "unmasking" of fixed –SH groups. For some time the phenomenon remained of chemical rather than biological significance, but with the claim of Anson & Mirsky in 1931 that protein denaturation is reversible, it entered a new realm of possibilities. And when it is remembered that among the agents which can cause denaturation of proteins, certain end-products of metabolism, such as urea (Hopkins[2]), are prominent, the significance of the fixed sulphydryl groups in Rapkine's scheme becomes apparent. The concentrations of urea required for protein denatura are of course very high, but it is by no means unreasonable to suppose existence of local accumulations in the cell.

The original rather crude form of Rapkine's scheme, then, was as foll (*a*) protein catabolism produces urea which accumulates at certain points wi the cell, (*b*) the urea brings about a denaturation of certain proteins, (*c*) the – groups arising therefrom reduce the soluble –SS– and change the oxidat reduction potential of parts of the cell towards the electro-negative side, (*d*) resulting partial anaerobiosis intensifies glycolysis (which may be norm proceeding), and with the energy so produced, cell-division is accomplished

The scheme as a whole was to some extent independent of its indivi components, for if it should be unplausible to regard the denaturation as du urea, there are many other methods (e.g. light, *p*H, hydration) by which it co be accomplished. Reversibility, again, was not essential, for a succession protein molecules might be envisaged. Nor the exact manner of reductio the soluble –SS–.

It was at first thought that the fixed –SH of the proteins was the only ag in the cell capable of reducing the soluble –SS–, but other systems are known to bring this about. Hopkins & Elliott could obtain reduction of glutathi by unknown substances in surviving liver. K. Kodama and Tsukano found soluble –SS– can be reduced by hexose monophosphate plus an enz extractable from heart tissue and adrenal cortex. Meldrum, again, reported intact red blood corpuscles rapidly reduce glutathione in the presence of glu and other hexoses. And Mann described a water-soluble enzyme from l which reduces glutathione in the presence of added glucose; in this case, howe other hexoses are inert. In the light of these facts, the association of soluble – with the reversible denaturation of cell proteins became less convincing. participation of carbohydrates in glutathione reduction, on the other hand rather striking, and there may now be, perhaps, a more plausible connec between –SH and glycolysis than between –SH and protein catabolism.

Whatever might be the exact mechanisms relating –SH to cell-divis further evidence of this relation continued to accumulate. In 1933 it was fo simultaneously by Rapkine[4,5] and by Dickens[1] that iodoacetic acid reacts stoich metrically with sulphydryl groups in the cell, whether free or fixed.* action of iodoacetate on sea-urchin eggs will be again referred to in the sect on embryonic respiration (p. 572); here it may be mentioned that Ellis failed to find any effect at weak concentrations, but later Runnström[17] obtai inhibition of respiration and blockage of cell-division using stronger ones. best of these studies is that of Rapkine[6] on yeast, who was able to show that a having inhibited all cell-division by carefully controlled doses of iodoacet the cells could be made to continue their division by the addition of glu thione. The different inhibiting action of iodine could also be reversed glutathione. Sachs, Rapkine & Ephrussi showed that the –SH groups of chi embryo extract could be blocked by iodoacetate, thus lowering its reduc power.

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* See also p. 204, where the effects of iodoacetate on the primary organiser in amphibia discussed.

Chalkley[2] later carried out some interesting experiments on the localisation of –SH groups in the amoeba, using the nitroprusside test. Most of the sulphydryl is fixed but nevertheless gives the reaction before protein denaturation; the proteins of the amoeba therefore resemble muscle protein (Mirsky[1]) rather than egg-white protein (L. J. Harris[1]). A strong coloration is always given by the cytoplasm, and a still stronger one by the nucleus, except during anaphase and early telophase. It appears that the sulphydryl is discharged into the cytoplasm at metaphase. In newly formed amoebae the nuclear reaction may not be stronger than the cytoplasmic one. Enucleation had no effect on either cytoplasm or nucleus.

The effects of the sulphydryl group are diverse. As may be seen from Hellerman's review, –SH groups affect the activity of many proteases. Glutathione is now generally regarded as the co-enzyme of glyoxalase (Giršavičius). According to Mayer & Voegtlin it inhibits nuclease, a fact which may be related to its effect on nuclear division. The association of glutathione with carbohydrate metabolism and dehydrogenases has recently been strengthened in several different ways; thus Hopkins & Morgan have shown that succinic dehydrogenase depends for its activity on the presence of sulphydryl groups, and Rapkine[7] found that triosephosphate and triose dehydrogenase is sensitive to sulphydryl groups. When oxidised by iodine, oxidised glutathione or dyes its activity ceases; when reduced by cystein, reduced glutathione or H_2S, it returns. The dehydrogenase activity can also be suppressed by copper and restored by H_2S. Iodoacetate inactivation, however, possible with very minute doses, is irreversible. Lastly, in the non-phosphorylating glucolysis of the chick embryo, there is a stage where sulphydryl groups appear to be necessary though glyoxalase is not involved (Needham & Lehmann[1]).

From the foregoing discussion it may be concluded that some connection probably exists between the sulphydryl group and cell-division. We come now o the work which has led to the suggestion that it is the intermediary between ne genotype and hereditary size limitation in the phenotype.*

It began by Painter's demonstration in 1928 that the size differences between 'olish and Flemish-giant rabbits are already perceptible in embryonic life (at 2 days' gestation), although the eggs are of exactly the same size. Painter found hat the Polish rabbit, which has an adult weight of about 1700 gm., had as 2th day embryo a length of 18·1 mm., while the Flemish-giant rabbit, which as an adult weight of about 5000 gm., had as 12th day embryo a length of 3·1 mm. He considered that the larger form had more cells and had carried ut more mitoses. Castle & Gregory confirmed that the eggs of differentized rabbits have identical dimensions (cf. the monograph of Pincus[1]). Taking bservations between 48 and 168 hr. development, they found that at 48 hr. he small race embryo had an average of 14·0 blastomeres, while that of the arge race had an average of 21·75. Similarly, the diameter of the blastocysts t 144 hr. was $40{\cdot}5\,\mu$ for the small race and $47{\cdot}8\,\mu$ for the large. Gregory &

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* A preliminary review has been given by Lerner[2].

Castle pushed this analysis further back still, to the 30th hour, wit following results:

Hours	Average number of blastomeres	
	Large race	Small race
32¼	4·41	4·06
40	9·94	8·29
41	11·64	8·62

Thus the difference persisted even into the earliest stages. Mitosis must be rapid in the larger races.

Estimations were now made of the glutathione (first by simple iodine tit and later by technique which allowed for the ascorbic acid present) in new rabbits of different size races (Gregory & Goss[1,2]). As Fig. 246 shows, constructed from the data in these papers, the larger the final size, the higher the glutathione concentration in the embryo at birth was found to be. This relation held good even when a Flemish-Polish cross was back-crossed to the large-size parent race, giving a point in the ¾ region[3]. That glutathione might have some connection with the pituitary growth hormone was suggested by the finding that the injection of growth hormone into adult rabbits which had stopped growing caused a rise in the glutathione content of their muscles, but no change in the ascorbic acid (Gregory & Goss[4]; Goss & Gregory[2]). This, however, would not apply to stages of development before the appearance of pitu function. There is also a lactation effect (Goss & Gregory[1]). It appears tha glutathione content of the new-born rat is greatly affected by nursing (e.g. n 56·0, not nursed 27·7 mgm. %); the figures on the diagram in Fig. 246 therefore obtained on non-nursed rabbits. When these figures were subj to allometric analysis (see p. 533) by Lerner, Gregory & Goss, differenc allometric constant were found as follows:

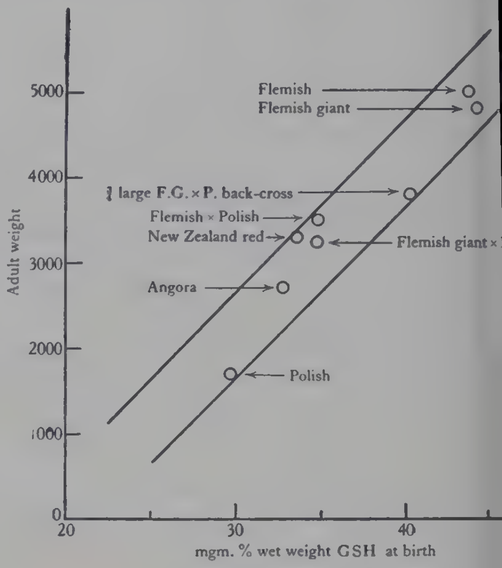

Fig. 246. Relation between size in ra and glutathione concentration at bi

Race	Adult weight gm.	Allomorphosis constant (see p. 53 glutathione
Flemish giant	4750	1·047
New Zealand Red	3300	1·255
Angora	2750	0·810
Polish	1680	0·617

This means not only that the larger embryos within a race have at birth glutathione than the smaller ones, but that this is more the case in the rac larger adult size than in those of smaller adult size.

Attention must now be given to the situation in birds. Byerly[3], who had working on small and large size races of chicks at the same time as the wo astle & Gregory on rabbits, had not reached similar conclusions. With White eghorns (adult wt. 2010 gm.) and Rhode Island Reds (adult wt. 2770 gm.) the mall size race was ahead of the large size one in weight before the 10th day of ncubation, after which the large size race drew ahead (weight measurements nly). Bucciante[2] had stated that within a given race the cell number is the same n chick embryos irrespective of the time taken to reach the stage in question, nd Milone found reasons for believing that in chick and sparrow embryos of he same morphological age the same number of cells is present. Measurements f this kind are particularly difficult to appraise. In conformity with Byerly, Henderson got no consistent weight differences between Dark Cornish and White Leghorn embryos at the same age.

Blunn & Gregory, however, considered that weight measurements alone were insufficient to settle the matter. Working with Rhode Island Reds and White Leghorns their results were as follows:

	Rhode Island Red	White Leghorn
Adult weight, gm.	3000	1560
72 hr. incubation: wt. mgm.	20·2	19·8
number of cells in the neural tube at a specified eye-region/embryo	61·6	57·5
mitoses/embryo	19·6	17·9
14 days' incubation: wt. gm.	12·08	11·37
number of cells, etc.	17·2	15·3
mitoses/embryo	2·14	1·68

Though the differences were statistically established, they are evidently nothing ike so large as the difference between the adult weights. Again, different conlusions were arrived at by Byerly, Helsel & Quinn, who studied the embryonic rowth of Single-Comb Rhode Island Red, Silkie, Single-Comb White Leghorn hicks, and their reciprocal crosses. Between 2 and 20 days of incubation Red mbryos and hybrid embryos in Red eggs grew at a practically identical rate. lkie and hybrid embryos in Silkie eggs also grew at an identical rate, lower than e former. Egg size seems therefore to be a more important factor than genetic nstitution in determining embryo size. The weights of the eggs are very fferent (Red 56·8 gm., Silkie 37·8 gm.). Embryo growth is thus adjusted mehow to the egg in which it is taking place. Cell counts indicated that the ur classes of embryos did not differ significantly from one another in cell umber.

Correspondingly with this difference between the development of mammals d birds, the results on the glutathione concentration were not as clear-cut in rds as in mammals (Gregory, Asmundson & Goss). No constant differences ould be detected between Rhode Island Red embryos and White Leghorn ones n glutathione concentration, though there was perhaps a very slightly greater mount in the former. But it seems that after hatching such differences begin to e perceptible, if we may judge from the comparison between Plymouth Rock hicks (adult wt. 2967 gm.) and White Leghorns (adult wt. 1988 gm.) made by regory, Goss & Asmundson, between 2 and 14 days of post-hatching developent. Here the glutathione concentration was consistently greater in the large size race, e.g. on the 6th day, Rocks 52 mgm. %, Leghorns 45 mgm. %. Gregory, Asmundson, Goss & Landauer measured the glutathione cont homozygous *Cornish lethal* and heterozygous *creeper* embryos throu development (see p. 382). The former are retarded in weight, the latter ar correspondingly the glutathione content was consistently lower than norm the former, and normal in the latter.

The views of Byerly, Helsel & Quinn were in agreement with the pre work of Kaufman[1,2]. She first found that the logarithmic weight/age c of hen and pigeon during embryonic life ran closely together, and tha Schmalhausen growth constants (*CE*, Section 2) were for the hen 346, fo pigeon 353. Thus between the 3rd and the 21st day of incubation the in taneous percentage growth rate of the two birds was identical, and coul account for the differences of body size. Even when the growth rates o individual organs were compared, the differences between the two birds wer slight. On hatching, however, large differences supervened, no doubt d genetic differences in nutritional efficiency and body proportions in growth. size difference was thus thrown back to the period before the 3rd day. Kaufn as the result of *ad hoc* cultivations of blastoderms *in vitro* and measuremen blastoderms of carefully determined age, believed that it lay in a difference i size of the cells themselves, so that even at the time of the appearance o primitive streak, the size difference was marked.* The primitive streak ap at the same time in fowls and pigeons, but its length in the two birds is as 1· Hence the volume, and probably the weight, would compare as approxim 2:1. In this case, therefore, we have to deal, not with a difference in the int tion of growth and differentiation processes, but with a difference in the attained by a cell before cleavage takes place. Kaufman[4] attributed a discord between her results and those of earlier authors (e.g. Levi) to the fact th drew his conclusions solely from linear surface measurements of blastode without taking into account the volumes.

Kaufman's work was all concerned with inter-specific differences, but Bye work on large and small breeds of fowls was amplified for large and small of pigeons by Riddle, Charles & Cauthen, who found that a part of the size differences is certainly expressed by the weights at hatching:

	Adult weight gm.	Hatching weight gm.
Runt	565	14·0
Homer	398	14·7
Magpie	296	11·5
Tippler	278	11·3

This would be expected because the eggs are of different sizes. But a large of the ultimate size differences in these races is attained through differential of growth after hatching during the first three weeks.

* On the 3rd day of development, the dimensions of a liver cell are, according to Kau for the chick 974 cubic μ, for the pigeon 596 cubic μ. On the 11th day they are for the 1223 cubic μ, for the pigeon 940 cubic μ. And no significant difference exists as regards freq of mitosis in the embryonic development of the two birds.

In sum, therefore, a definite difference appears to have been established between lutathione concentration of large and small races in mammals, but in birds this s not seen until after hatching. Before hatching, the size of the container is aramount. We cannot but be reminded of the discussions which have taken lace about the metabolic peculiarities of the cleidoic egg (e.g. *CE*, p. 1613 nd above, p. 48), the difficulties of nitrogen catabolism and the general deelopmental acceleration of birds over mammals. Even in mammals, however, ome kind of special size limitation related to the size of the container comes nto play when a large size race embryo is caused to grow in a uterine environnent intended only for small size race embryos. In the interesting experiments f Walton & Hammond the large Shire horse (adult wt. 800 kg.) was combined ith the small Shetland pony (adult wt. 200 kg.) in reciprocal crosses by artificial nsemination. At birth the weight of the foals was found to be proportional to he weight of the mother and equal to the weight of foals of the pure breed of he mother, so that maternal regulation of foetal growth had completely obscured he expected genetic effects:

	Birth weight (kg.)
Pure Shetland	20
Shetland ♀ × Shire ♂	19
Shire ♀ × Shetland ♂	50
Pure Shire	59

fter weaning the growth rate established itself as rapid or slow and the characeristic size differences were gradually attained. The mechanism of this regulation emains entirely unknown. But as it is known that the weight of individual rabbits n a litter is inversely proportional to the number produced (Wishart & Hammond; Iammond[1]), although there is room in the uterus for a larger volume of foetal aterial than is actually there, some regulation of foetal nutrition, possibly ndocrine in nature, may be suspected.

The difference between the experiments on rabbits and those on horses lies rhaps in the fact that Gregory and his collaborators were studying a narrower nge of birth weights than Walton & Hammond. The difference between the allest and largest rabbit birth weights seems to be about 1 : 1·57, but that tween the small and large size horse birth weights is 1 : 2·75. Were birth-weight fferences in rabbits sufficiently extreme they might, in the case of crosses, mask maternal regulation of size the genetic control of size and what seems to go ith it, the glutathione concentration. Though range of birth weight will hardly count for the "maternal" regulation seen in the development of the chick, e conditions of embryonic life are there so much more rigid that the regulation ight be expected to function at a lower level of hatching-weight differences atching weights: Silkie 24·4, Rhode Island Red 33 gm.).

A glance at a double-logarithmic plot showing birth and hatching weights of any mammals and birds (*CE*, Fig. 72) will show how extremely narrow are the nges which have so far been studied.* Nevertheless, it may well be said that

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What shall we say of size-control in the fishes where the parasitic male is extremely minute, or ants such as *Carebara* where the queen takes with her, as workers to help in founding the new lony, individuals a thousand times smaller, attached to her legs?

the glutathione effect could only be detected on races likely to be of cl similar chemical constitution.

Before quitting this subject it is worth while to note that among the inv brates and prochordates there is sometimes a remarkable constancy in cell cell number, and body size, as in the rotifers, nematodes and trema (H. J. van Cleave) and the ascidians (Berrill[5,6]). Nothing is known regardin physiological mechanisms underlying these phenomena.

This completes the consideration of the border-line between embryolog genetics. There remains the problem of regeneration and the study of orga effects in invertebrates.

2·7. Problems of later amphibian life-history

Before ending the discussion of the experimental morphology of verteb and dealing with that of the best analysed invertebrate groups, it seems i pensable to say a few words about regeneration and metamorphosis. The ph mena of regeneration, as of limbs or tails, in vertebrates, are important be they raise the following questions. How far may such regenerations be rega as repetitions of ontogenetic processes which have already occurred? Is termined differentiation preceded by a period of plasticity or pluripote What sort of biochemical processes are proceeding in regeneration and do bear any relation to those proceeding during normal development?

2·71. Regeneration, determination, and the organ-districts

The problem of regeneration has already been met with in this book. The pres or absence of regenerative power seemed to be of significance for the ca problem (p. 240); regeneration phenomena were referred to in the quotation Spemann on organ "fields" or districts (p. 286); and the misgivings abou concept of determination voiced by Harrison (p. 110) were partly due to the appearance of pluripotency which has been believed to occur in regenerati

The aim of the present section is to describe the most important facts an give a critique of the views which have been based upon them. As in the of genetics, an exhaustive bibliography will not be required for this purpose, outlying regions of the very extensive literature can be reached through interesting reviews of Weiss[4,11,15,16,17]; Guyénot & Ponse[2]; Woerdem Poležaiev[3,7,9]; Waddington[8]; Schotté[16] and the book of Korschelt.*

Whether or not regeneration of a limb, for example, occurs in an adul nearly adult amphibian, has been ascribed (p. 240) to the presence or abse the persistence or disappearance, of the former Individuation Field of the emb As we have seen (p. 288), the main field splits up at the neurula stage a number of organ districts,† within which the various structures of the a ody arise. This emancipation of the districts is still seen in regeneration; he adult individuation field is such as to ensure that an organised entity is egenerated, but the character of what that is depends on the stump and ot on the body as a whole. This was first proved by Guyénot[2] and by Weiss[1], o whom we owe a large part of our knowledge of amphibian regeneration. f a fore-limb of, for instance, a salamander larva, is transplanted homoiolastically to the region of the hind-limb, or *vice versa*, and the transplant, nce well healed into place, is partly re-amputated, what will be formed will epend on the stump and not on the region of the body into which the tump has been put. The stump, not the plan of the body as a whole, controls he character of the regenerate. Fore-limb stumps in hind-limb district give ore-limbs; hind-limb stumps in fore-limb district give hind-limbs; the stump an therefore "carry the field with it" (Weiss[1]; Ruud). Similarly limbs translanted to the back or flank will regenerate limbs there (Milojević), or tails will egenerate tails in similar improper and teleologically useless situations (de Giorgi & Guyénot) (Fig. 247, case (1)).

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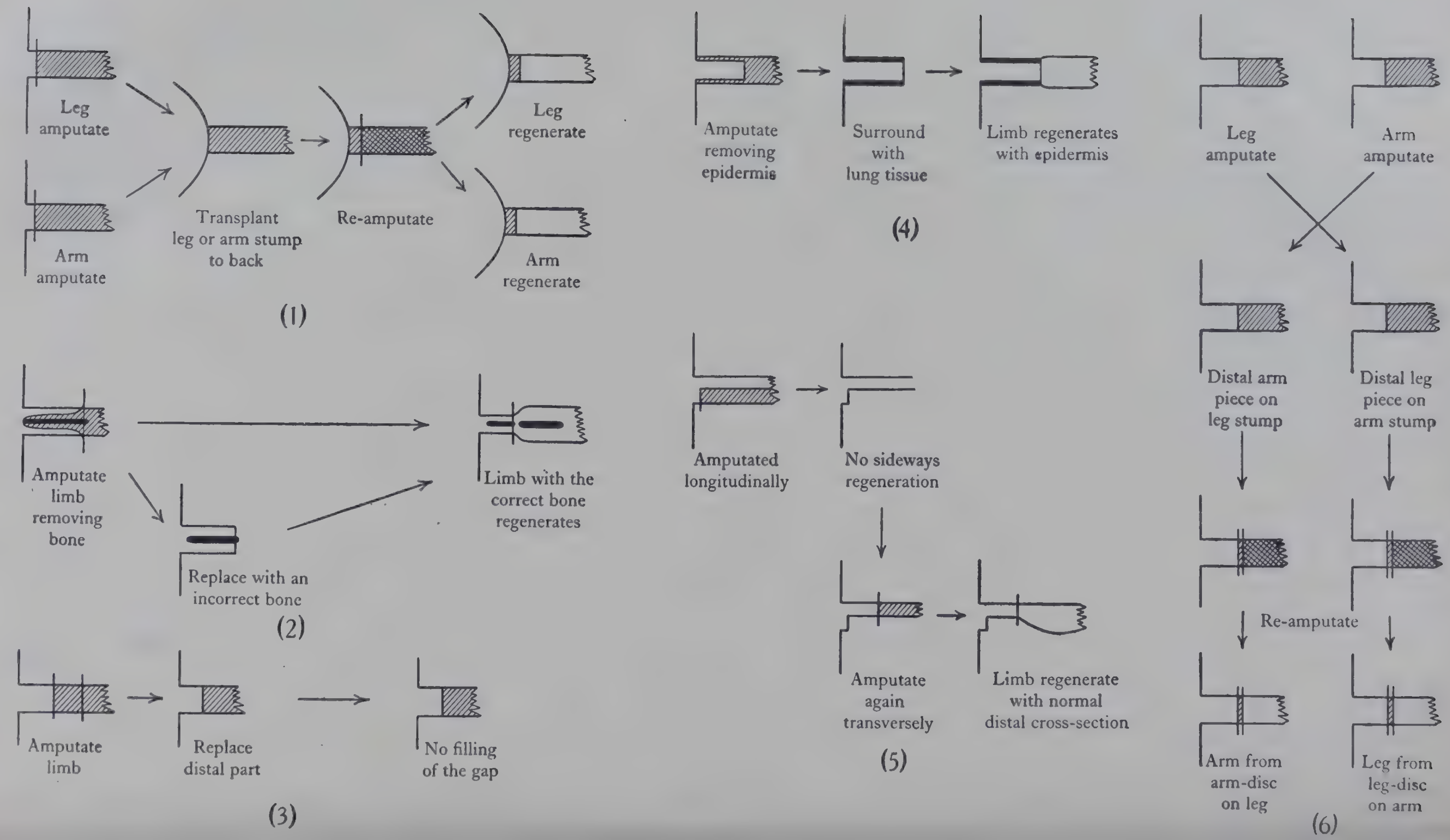
* I exclude from consideration the regeneration of invertebrates (for which see the revie Korschelt; Huxley & de Beer[2]; Stolte[2]) because for us the interest of regeneration lies i relations to induction, determination, competence, etc. Practically nothing is known about things in invertebrate development, with the exception of that of the echinoderms. problem of determination in maturing mosaic eggs will hardly be solved until our knowl of its more striking features as seen in regulative development is further advanced.

† The same, I shall assume, as Guyénot's "territoires de régéneration".

There are also limits to what the body can regenerate at any one time; if too uch material be taken away, regeneration fails. The organ districts, in fact, are uite sharply delimited. For example, Schotté[2] found that in *Triton* the removal f the tail anterior to the last sacral vertebra would prevent its regeneration, hough it will regenerate when the cut is at any point posterior to this. This ill be referred to again (p. 435). Here it need only be said that if the volume of he regeneration blastema is substantially reduced, deficient morphogenesis, such s suppression of digit formation, will follow (Guyénot & Schotté[1]). Yet on the ther hand regenerating blastemas can be fused so as to produce single well-haped limbs until quite a late stage in the regeneration process (Swett[1]; Weiss[1,3]; ilojević & Vlatković). The whole process is, indeed, a remarkable mixture of egulative and determined development.

Its limitations are as instructive as its sometimes noteworthy powers. It can generate only the whole structure; not individual components. If the skeletal rt is removed from a limb, the bones are never regenerated, but the gap is filled ith connective tissue (Schaxel & Böhmel). If now, however, the boneless limb amputated, regeneration of a perfectly formed limb, *including* the skeletal parts, llows from the boneless stump (Weiss[2]; Bischler; Liosner, Voronzova & ozmina). Similarly, if the bone which is removed is replaced by bone from nother kind of limb, the limb which regenerates distally after amputation has evertheless the skeleton characteristic of the original one (Weiss[2]; Bischler & uyénot) (Fig. 247, case (2)). A femur implanted into an arm from which the umerus has been removed will have no deviating effect whatever on the arm-uality of the subsequent distal regenerate. Gaps in a limb cannot be filled in Schaxel[2]) (Fig. 247, case (3)).

Similar experiments have been made as regards the skin. Weiss[6] removed the idermis from an amputated stump and encased it in a sleeve of lung tissue, hich prevents the appearance of epidermis again upon the stump. Nevertheless, e limb which regenerated was well provided with normal epidermis (Fig. 247,


Leg amputate
Arm amputate
Transplant leg or arm stump to back
Re-amputate
Leg regenerate
Arm regenerate
(1)
Amputate limb removing bone
Replace with an incorrect bone
Limb with the correct bone regenerates
(2)
Amputate limb
Replace distal part
No filling of the gap
(3)
Amputate removing epidermis
Surround with lung tissue
Limb regenerates with epidermis
(4)
Amputated longitudinally
No sideways regeneration
Amputate again transversely
Limb regenerate with normal distal cross-section
(5)
Leg amputate
Arm amputate
Distal arm piece on leg stump
Distal leg piece on arm stump
Re-amputate
Arm from arm-disc on leg
Leg from leg-disc on arm
(6)

case (4)). Taube's experiments were even more striking. He replaced the black skin of the normal limb by a sleeve of red skin from the belly, and when this had properly healed, amputated the limb in the middle of the red portion. The regenerate, which appeared normally, was covered with black skin, not red. This has been confirmed by Holzmann. All these facts go to show that the tissues of the stump do not each give rise to the corresponding tissues of the regenerate, but rather that relatively undetermined mesodermal cells come forward and are organised into everything that the new structure needs, and in accordance with the "style" of the old. Exactly how undetermined they are is the most important point of all; we shall return to it before long.

Another remarkable limitation of the regeneration process is that it only works in a distal direction, not sideways. If a limb is amputated not transversely, but longitudinally, a certain amount of growth will subsequently occur on the injured side, but there will be nothing like a completion of the normal cross-section of the limb (Weiss[3]; Gräper[1]). On the other hand, if such a split limb be amputated transversely, a distal regenerate will be formed which will have a normal cross-section (Weiss[5]) (Fig. 247, case (5)).

The quantity of stump material which can carry the limb district quality is remarkably small (Milojević). A thin transverse slice of fore-limb, for example, healed on to the stump of a hind-limb, will give rise to a fore-limb regenerate (Weiss[1]) (Fig. 247, case (6)).

In the foregoing paragraphs it has been said that the complete regeneration process seems to work only distally. If the stump is turned round and healed into place so that regeneration would have to occur from the proximal part of the limb or not at all, only replications of the stump itself occur, and not regenerations of the entire limb. Thus if a knee, with a piece of upper and lower limb, is inverted and implanted into the body so that the upper limb end protrudes, a new knee and lower limb is formed, but never anything in the nature f a foot (Kurz; Gräper[1]; Milojević & Grbić). The same effect has been observed n inverted pieces of tail implanted into the dorsal region (Milojević & Burian). uch phenomena go far to explain many spontaneously occurring limb abnorıalities (Przibram[3,6]; Puppe and others).

From the facts so far described it might appear that it is impossible to influence he organ "field" itself; if it works, it works in a unitary way leading to a complete eplica of the original structure. Nevertheless, Vogt[4] showed that larvae with efective tails caused by injuries to the embryo in the earlier "mosaic" stage partial extirpation of the organ district) would subsequently regenerate defective, ot whole, tails. Something similar to this occurs in fishes (Nabrit).

It is interesting that the presence of the nervous system is absolutely necessary or the regeneration process; complete severance of the peripheral nerves in the imb region of urodeles leads to failure of regeneration, as was proved, after some ivergence of opinion, by Schotté;* and complete de-differentiation of the

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This was first discovered by Locatelli, but acceptance of the fact was delayed by her erroneous elief in a specific morphogenetic influence of the nerves. Trophic action of the nerves seems to revent de-differentiation going too far.

stump (Schotté & Butler). Partial denervation slows the regeneration proc and late denervation arrests it halfway. It needs the presence of the neural tu and the sympathetic system, but can go on without continuity of the m segmental motor and sensory nerves. Whatever the nature of the nervous fluence is,* it cannot override the stump-quality, as the facts already descril demonstrate. By deviating the course of nerves to the various organ distri corresponding regenerates or new supplementary growths can be produced in the experiments of Guyénot & Schotté[2]; Guyénot & Ponse[2]; Bovet, e but the effect of the nerve is always trophic rather than determinative (Scha & Schneider; Samaraiev). Traces of such a trophic action have, indeed, be reported for normal embryonic limb-buds, whether *in situ* (Hamburger[3]) transplanted (Hamburger & Waugh).

Alone of the organ districts the crest "field" has been successfully stimula mechanically. On tying a thread round the body to get mechanical duplicat of limb blastemas Milojević, Grbić & Vlatković found in a few cases a grov of crest in the neighbourhood of the thread, which was sewn through the sk This phenomenon is probably not very important.

The question of the origin of the tissues in the regenerated limb, to whi reference has already been made, long presented much difficulty, but was eve tually solved by the ingenious experiments of G. Hertwig[2]. If a haploid arm transplanted to a diploid animal, and then after healing into position, amputat the resulting regenerate is entirely composed of haploid cells. The source of t cells must therefore have been the de-differentiating stump and not the rest of t body.† Evidence which supports this conclusion, without being in itself decisi comes from experiments in which arms of the black axolotl, transplanted to albino axolotls, regenerate black arms, not white, after amputation (Ruu Similarly, *Triton* limbs on *Salamandra* hosts, recognisable by differences nuclear size, regenerate limbs of their own character, not even intermedi between that and the character of those of their hosts (G. Hertwig[2]). Eviden clinching the matter comes from radiation experiments (see below, p. 440). T histology of the regeneration process has been exhaustively described Naville[1] and Thornton.

Accurate knowledge of the normal rate of regeneration is available. We o to Grotans[2] an elaborate study of the speed of regeneration of amputated axol gills. At first it is rapid, then for several weeks it continues at a steady rate, a thereafter it regularly falls off. With increasing age, the initial rise is slower, t maximum less persistent, and the final fall more gradual. The figures of Murtas however, show that the speed of regeneration of these axolotl gills temporar increases again after a certain minimum point is passed. Syngajevskai measurements for *Siredon* limbs indicate that their speed of regeneration maintained constant over a remarkably long range of body weights. And simi quantitative comparisons between the growth of the axolotl tail in norn development and during regeneration have been made by Godlewski & Latinik-Vetulani. The surprising fact is reported by many observers that regeneration of one extremity (tail or limb) is *accelerated*, not inhibited, by the amputation of another shortly afterwards (Zeleny[1]; Hiller[1]; Blacher, Irichimovitch, Liosner & Voronzova).

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* Could it be an effect of sympathin? (see p. 211).

† This is in some contrast with conditions in invertebrates, where there appears to be a migrat of "reserve" cells from all over the body to the blastema (see the review of Stolte[2]).

With the question of the state of determination of the blastema we enter a debatable land. Is a limb blastema determined from its very first appearance to form a limb, and a tail blastema to form a tail, or can their fates be shifted by implantation into a district foreign to their normal fate? Is their behaviour *ortsgemäss* or *herkunftsgemäss*? It has become customary to say that in their youngest stages they may show *ortsgemäss* (neighbourwise) behaviour; i.e. that they are at first pluripotent or relatively undetermined.

The facts upon which this view rests are the following. From his transplantations of young blastemas between fore-limb and hind-limb districts on the same animal Milojević concluded that if a blastema was placed, while still sufficiently young, in a foreign district, its fate could be changed, and a presumptive hind-limb would become a fore-limb (in contrast with experiments where the old stump is transplanted as well). As there might be difficulties in deciding whether a rather abnormal regenerate was to be regarded as fore- or hind-limb, and in order to see whether the pluripotence was really wide, Weiss[7] proceeded to exchange presumptive tails and limb blastemas. He obtained the *ortsgemäss* effect, a limb from a presumptive tail blastema. The converse experiment was successfully performed by Guyénot & Schotté[3]. Proof of pluripotence appeared to have been given.

But the case is not really as convincing as has been supposed. The number of successful reversals of presumptive fate was small; Weiss described only three, and Guyénot & Schotté only one. True, others were later adduced by Schaxel[4]. Much more serious is the fact that very young blastemas show a marked ndency to resorption. As Weiss himself points out ([16], p. 107), a blastema less than 2 weeks' development, transplanted to an indifferent situation, such the flank, is always resorbed. The possibility exists, therefore, that what happens the cases of apparent reversal of presumptive fate is a resorption of the young astema, followed by the normal regeneration of the typical local structure. In is case all regeneration would be *herkunftsgemäss*. Now the most obvious ay of preventing resorption would be to implant, not one tail blastema, for xample, but several, on to the site of an amputated limb; in this way the danger resorption would be minimised and, if a change of fate occurred, pluripotence ould be placed on a much surer basis. This experiment was carried out by oležaiev[2], with decisive results against pluripotence. With one blastema he was ble to confirm the apparent change of fate, but when he implanted from four eleven blastemas the results were without exception otherwise.* Tail blastema

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The fusion of several identical or analogous embryonic parts into one for analytic purposes has en a valuable contribution by the Russian school, cf. the work of Lopaschov[2] already mentioned 283) on putting together several primary organisation centres. It derives perhaps from the servation of Filatov[4] in 1932, that by combining several tail blastemas into one the speed of generation of the tail could be greatly accelerated.

material, if present in sufficient amount to guard against resorption, invar produced tail though in the limb district.* This was later confirmed by Liosı

Poležaiev[5] also showed that the tissues of a limb may be thoroughly mi and will yet, when implanted, regenerate a well-formed limb.† In later ex ments he found that a limb will regenerate *herkunftsgemäss* if to one blast itself incapable of self-differentiation, is added a mass of minced mesoderm other blastemas, even from young ones themselves incapable of self-diffe tiation. Resorption must be regarded as due to the lack of a source of mesode cells, undetermined within the limits of the limb as a whole, which can give material required for regeneration. In the earliest stages, this source i course, the de-differentiating stump.

A further contribution to the determination problem was made by Lodyžens using the method of antero-posterior and dorso-ventral inversions of transpla limb blastemas. Contrary to the impression gained by other workers, suc Milojević and Weiss, she found that the blastema's spatial dimensions already determined during the early part of the first week of regeneration. spite of the much fuller later work of L. David and Schidewski, conclusions are difficult owing to the resorption process. The general result is neverth reached that wide pluripotence probably does not occur in regeneration, and each organ district in amphibia is capable, if the individuation field is still tact", of reproducing the organ in question.‡ Material from such districts ca be made to form organs characteristic of other districts. That regeneration sh not reproduce the condition of wide pluripotence seen in early embry development is not after all so very surprising, for, as we have seen, it depe on certain factors quite foreign to the embryo, such as a connection with nervous system.§ It is nevertheless still necessary to withhold a final judgm since Efimov[5] has reported three cases of exchanges between young hind-l blastemas of the black axolotl to fore-limb site in the white axolotl, in which resulting regenerates were black fore-limbs, suggesting that a change of presumptive fate had taken place. It might, however, be said that only the epidermis, or only the melanophores, had been derived from the transplanted blastema, while the remaining tissues were of host origin. Moreover, Savchuk could not repeat Efimov's results, and in new elaborate work Mettetal always obtained *herkunftsgemäss* differentiation in salamanders.

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* This whole subject has been the cause of a good deal of unfortunate terminology. Weiss[11] Schaxel[3] have used the word "nullipotent" to describe the status of the very young blastema on transplantation to an indifferent site is resorbed and performs no morphogenesis. But all could be said of it was that it was "not capable of differentiation" and hence its potencies not be tested; hence the word "nullipotent" begged the issue. Since Poležaiev's work[2] we sh call it "not capable of self-differentiation" and neither nullipotent nor pluripotent. The onl for the word nullipotent that I can see is to describe such tumours as cannot be made by known means to perform any morphogenesis but which nevertheless proliferate. Pole himself has also erred, however, for he uses the words "undetermined" and "determined describe the status of the blastema before and after the onset of the capacity for self-differentia Since nothing whatever, owing to the resorption difficulty, has as yet been found out abou status of the blastema before self-differentiation is possible, this use of the determination co is unjustified.

† Poležaiev recalled in this connection the reunition of sponges after the destruction of organisation by filtration (see p. 528).

‡ Regenerative capacity may be remarkably great; according to Schaxel[4], twenty-two regenera by one animal are possible.

§ Weiss' pluripotence experiments[7] have also been subjected to another criticism by Guy and Bovet, who pointed out that in the implantations of the tail blastema on to the limb sit interference with the nerve is a serious source of error, for this alone might be expected to pro a regenerated limb from the host's limb district.

The question may next be asked: What part do the various tissues of the regenerate play in determining its nature? Important qualities seem to be carried by the epidermis, for Efimov[1] earlier found that if epidermis of the amputated limb stump is replaced by head epidermis, no regeneration ensues (cf. p. 304). It had previously been shown by Tornier[2] and by Godlewski[2] that if an amputation surface is covered *immediately* by any skin, regeneration is inhibited. Efimov[1] then showed that such an inhibition only occurs if the skin is put on before any natural epidermis has had time to form; the effect is therefore by no means mechanical. This was further extended by Poležaiev & Favorina, who found that replacement of limb epidermis by head epidermis at any time during the regeneration process would inhibit it, though naturally as the regeneration proceeded the inhibitory effect became less marked. Poležaiev & Favorina also showed that the removal of the regenerated epidermis alone would itself stop regeneration; a skinned blastema will neither grow nor morphologically differentiate, though its histological complexity may increase (Fig. 248). A close connection between mesoderm and epidermis is therefore a *sine qua non* of regeneration. Correspondingly, I. I. Morosov has shown that if after inhibition of regeneration by skinning, the blastema is put under the skin of the tail, regeneration will proceed. But the longer the period of inhibition has been, the more abnormal are the resulting structures. In the early stages of the regeneration, the action of the epidermis is supposed to be a histolytic one (Poležaiev[1]).*

The mesodermal parts are also very important. Liosner & Voronzova[3], after amputating the hand of an axolotl, dissected the arm and replaced the whole of the arm musculature with a muff of muscle taken from the tail, retaining the nerve and blood-vessel. In many such cases regeneration almost led to the ransformation of the structure into a tail, but sometimes structures intermediate etween tails and limbs were formed. It is therefore certain that the morphoenetic process of a regenerating limb may be caused to deviate in the direction f tail if tail muscle is present instead of limb muscle.† Liosner & Voronzova[5] escribed one successful case of the converse experiment. Subsequent work has hown that tail muscle transplanted into a bone-free limb can form fairly typical ail skeletal parts (Liosner[3]). Under such conditions the approximation of the egenerate to a tail is much more complete; the limb bone therefore exerts a imb-quality-retaining influence (Liosner[4]). Just as the muscle mesoderm of a imb blastema can be replaced by tail muscle, so it can be replaced by muscle rom various proximal-distal levels of a normal limb. When this is done, the egenerate is found to have a corresponding character, i.e. if muscle from the distal end of the limb is inserted, hands and feet only are regenerated; if muscle from the shank portion of the limb, a full limb is formed (Voronzova[1]). Recip transplantations of fore-limb and hind-limb muscle into young regene demonstrated the same kind of specificity; the resulting structure depend a considerable extent on the origin of the musculature which had been put (Voronzova[2]). If musculature from the dorsal part of the body is put int regenerate instead of its own muscle, the results are variable; regeneration fail, the fate of the regenerate may be unaltered, or minor modifications of it occur (Voronzova & Krascheninnikova). Bones of the shoulder girdle ca regenerated from shoulder-girdle musculature, even in abnormal situation young larvae (Voronzova[3]). And cartilage of a new limb skeleton can arise fr blastema in which there are no cells of cartilage origin (Thornton).

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* Adova & Feldt have attempted to establish chemical differences between the skin of the back, which will not take part in leg regeneration, and that of the limb, which will do so. The autolytic process was found to differ.

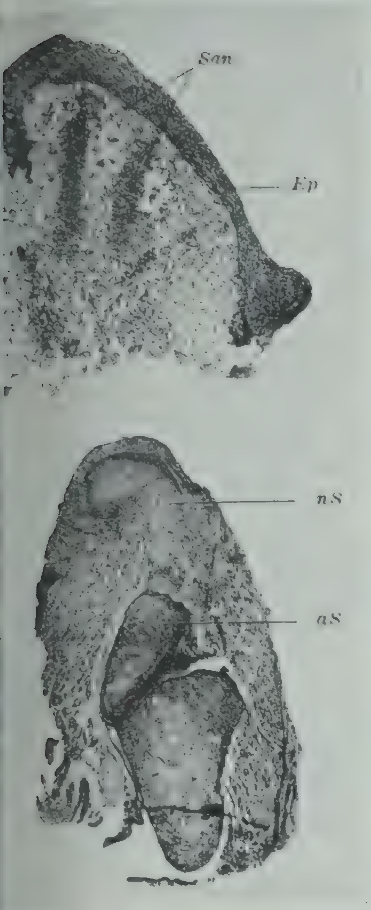
† But though the tail implant will make the surrounding presumptive limb tissues behave eighbourwise as regards their morphogenesis, they behave selfwise as far as the stage in the lifeistory is concerned. In other words the tail inductor may be from an adult, but the tail produced will have larval characteristics if regenerating on a larva (Voronzova[4]).

In tail regeneration Okada and others have shown that the presence o spinal cord is very important in the determination of normal cross-sectio it is destroyed bone may appear and may differentiate histologically, but vertebral organisation will not be formed. Without the axial organs a m finlike structure regenerates.

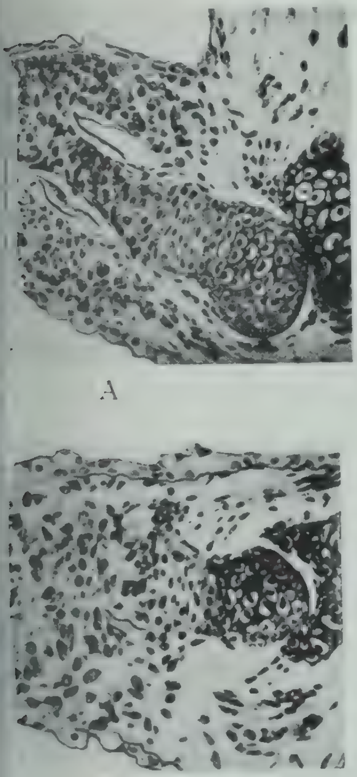
The part played by bone in the regeneration of a limb has been consider Liosner[6]; and Nassonov's work has indicated that the action of cartilage must not be neglected. First he obtained[1], like Poležaiev[8], duplication of li and appearance of digital processes, etc. by implantation of regeneration blast destroyed by mincing. He then found[2] he could obtain the same result implanting regeneration blastemas dried at low temperatures, in which the had all been killed, though here the number of positive cases was smaller. supported the earlier view that an important role in regeneration is playe the decomposition products of the traumatised tissues. Next he found tha addition of an aqueous extract of cartilage would, when added to a tissue cul of connective-tissue fibroblasts, cause the formation of a large amount of hy cartilage, sometimes even converting the whole of the culture (Nassonov[3]) (Fig. This could not be confirmed by Rumjantzev & Berezkina (see above, p. Finally, he found that when implanted under the skin in the limb district o axolotl, a piece of cartilage would give rise to an epidermal prominence resem a regeneration blastema, as shown in Fig. 250 (Nassonov[4]). Further organisa however, did not generally occur (cf. the ectodermal proliferations obtaine implantations of chemical substances into the blastocoele cavity, p. 177).

In subsequent papers Nassonov[5] showed that the morphogenetic powe cartilage was destroyed by boiling (confirmed by Kozmina) and that carti from anura but not from reptiles or mammals was active. Epidermis, bone muscle tissue was not, but lung, small intestine and gill showed conside activity[6]. The products of an alkaline hydrolysis of cartilage can produce digital appendages with cartilage within them. Among the interesting feat of this work (continued by Peredelsky) is that it recalls the induction of buds in the embryo by ear-vesicles and nasal placodes (p. 305; Balinsky[6]) tho the phenomena in the adult are much less striking morphogenetically.

The cause of the naturally occurring loss in regeneration power (referre above as disappearance of the individuation field) is interesting but obscure. the urodeles, as has been known since the 18th century (Barfurth[2], 1895), a st power of regeneration persists into adult life, but the anura can only regene their limbs at the early stages of metamorphosis. Afterwards the power is It seems, indeed, to arise and to disappear in a very definite way. The wor Svetlov[1] and Veizman on anuran tadpole-tail regeneration shows that in the



Inhibition of amphibian limb-
ation by removal of epidermis:
nal blastema; (*b*) skinned blas-
San, skeletal rudiments; *Ep*,
his; *nS*, newly forming bone;
nains of old bone.



A

B

hibition of amphibian limb-
ion by X-rays. A. Normal
B. Irradiated blastema in
differentiation of the carti-
ite inhibited.

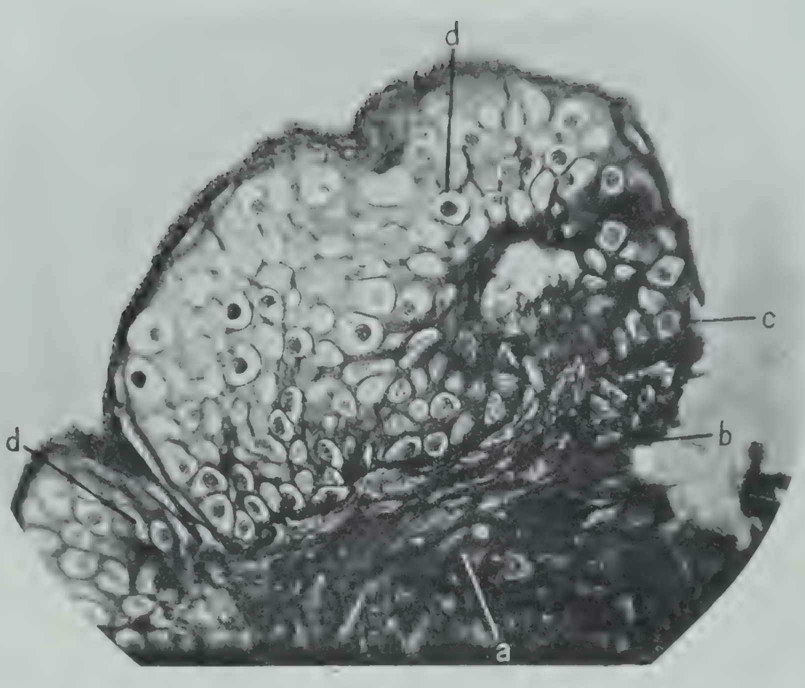


Fig. 249. Induction of cartilage from fibroblasts by cartilage-extract (Nassonov). *a*, connective tissue fibroblasts; *b*, transitional zone; *c*, rounding fibroblasts; *d*, cartilage.

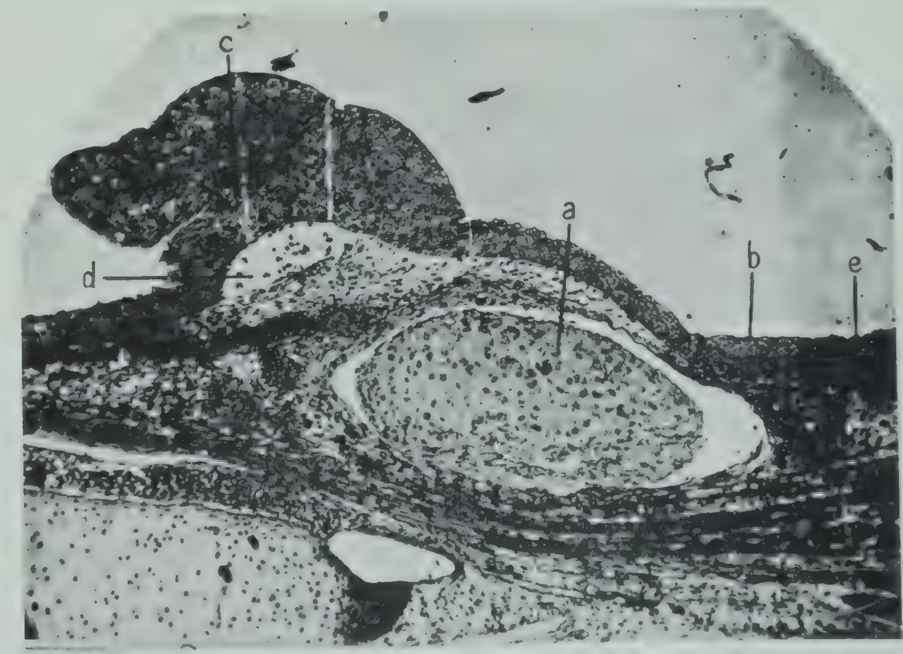


Fig. 250. Induction of epidermal prominence by implantation of cartilage (Nassonov). *a*, disintegrating implanted cartilage; *b*, epidermis; *c*, epidermal outgrowth; *d*, cavity containing blood.

tail-bud stage no regeneration is possible, but that the power reaches a maximum about the time of hatching from the egg-membranes. Before metamorphosis it has disappeared. In lizards the tail can regenerate but the limbs not;* Weiss[10] found, however, that regeneration could occur in the limb district if a tail blastema was implanted there. It was strictly *herkunftsgemäss*.

Poležaiev's experiments[4] on limb regeneration are also interesting. By traumatisation of the wound surface (mincing it with a scalpel *in vivo*) he found it possible to obtain limb regeneration at a stage subsequent to that at which the power normally disappears. He explained this by assuming that the traumatisation stimulates the accumulation of the indifferent regenerative cells from which the blastema is formed, and that it is the lack of these which is involved in the normal decline of regenerative power. He showed also that re-regeneration (the regeneration of a limb that has been itself produced by regeneration) may take place quite late in metamorphosis. These facts would indicate that the mechanism of the loss of regeneration power has something to do with the availability of the relatively undetermined mesoderm cells (Poležaiev & Ginzburg). But this does not take us very far.

Much of the discussion about loss of regeneration power has, of course, turned upon the question of whether this loss is brought about by a humoral change in the whole body, or whether it is localised in the limbs. Thus Guyénot[1] first showed that tails and limbs of young frogs which had lost their power of regeneration did not regain it when they were transplanted on to salamander larvae in the proper positions, although salamanders never lose regenerative power. Transplanted and then amputated, they never grew again. Liosner[2] and Naville[2] completed the picture by transplanting backwards and forwards in time (cf. p. 346). From frogs that had lost the power, limbs were transplanted to frogs which still had it; and *vice versa*. In this case also, the limb behaved physiologically *herkunftsgemäss*. If it could regenerate before, it would do so just s well on an older body; but if it had lost the power, transplantation on to a ounger body, where the power was still present, did not restore it. All this eems to prove that the change is a local one. But if this is really so, limbs from adpoles at the regenerative stage, transplanted on to young frogs just about to ose their regenerative power, should lose their regenerative power later than the mbs of the host. On the other hand, if the body as a whole were concerned, the power should be lost in all the limbs approximately simultaneously. This xperiment has been tried by Borssuk, with the result that the second of these lternatives was found to hold good. So it would seem that both local and general hormonal) factors are involved. This agrees with the fact established by Poležaiev[12] that the conditions under which the tadpoles are maintained affect the time at which the regenerative power falls off.

That hormones are involved in amphibian regeneration appears from many experiments, such as those of Schotté[1] on the pituitary and Speidel on the thyroid. Hypophysectomy suppresses regeneration in *Salamandra* larvae and adult *Triton*

* There is a very minor degree of regeneration of lizard limbs according to Guyénot & Matthey.

but not in larval *Triton*. The effects of thyroxin are complex.* But if the ci lating hormones are in any sense inductors here, the competence of the tis must vary independently, for we know that the power of tail regeneratio toads, for example, persists for some time after the power of limb regenera is lost (Guyénot[1]).

In mammals, especially embryos, traces of regeneration power can be obse if we may accept the results of Mitzkevitch and Selye (on limbs) and of Sely McKeown[1] (on the uterus).

The loss of regeneration power normally occurring in some animals can we have seen, be stayed by certain methods. Conversely, it is possible to inh regeneration in animals at stages when it is normally vigorous. Inhibition withdrawal of the trophic action of the nervous system has already been m tioned. Moreover, if the stump is covered with epidermis immediately a amputation, no blastema will form (Tornier[2]; Godlewski[2]). Or if too gre quantity of material is taken away, regeneration will be inhibited. Thus if whole of the shoulder girdle in adult newts is removed, no regeneration foll (Weiss[2]), though in younger larval stages this regeneration is possible (Swe Swett & Parsons) and will even go on in unlikely situations such as the hi limb if the shoulder muscles are transplanted there (Voronzova[3]). There is age series of graded effects (E. H. Parsons). But the most controllable way inhibiting regeneration is by the action of radiant energy which can be fa accurately dosed.

As we learn from the interesting review of Curtis, it was Schäper in 1904 discovered that exposure to radium emanation would inhibit limb regenera in *Triton* larvae. After this initial observation nothing was done for thirty y until a series of papers by Butler[1]; Liczko; and Brunst & Scheremetieva clea up the question and showed that the inhibitory effect of X-rays may be us in studying the nature of the regeneration process. Ultra-violet irradiation inhibits it (Tolmatcheva-Melnitchenko). In either case, stump de-differentia will follow if the irradiation is applied early (Butler & Puckett) or the regenera will be arrested midway if it is applied later.

In a normally regenerating limb the first component of the blastema differentiate is the cartilage. In an irradiated larva not only is there no indica of cartilage differentiation, but also the old cartilage within the stump is seve affected by the radiation and finally disappears (de-differentiation).† Butler[2] c cluded that the primary effect of the X-radiation was to inhibit the histolog differentiation of cartilage. Fig. 251 (opp. p. 438), from Butler, illustrates his resu Liczko's interpretation of what happens was a little different, for he regarded primary effect as the formation of a mass of mesodermal connective tissue at distal end of the blastema into which the cartilage and muscle cells were un to penetrate. Whatever the effect is, it will persist, as Liczko found, in the tiss and will manifest itself if an amputation is made as long as 8 weeks after the irradiation.

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* According to B. D. Morosov, mammalian or amphibian embryo extract, injected int amphibian body-cavity, accelerates tail regeneration.

† Since the effect of irradiation is analogous to the cutting off of the trophic action of the pathetic nervous system, may there not be a common point of chemical action here?

The effect is local, not general (Butler[2]; Brunst & Scheremetieva[1]). Irradiation of a single limb will prevent its regeneration, and an unirradiated limb will still retain the capacity for regeneration though a large part of the body has been irradiated. Unirradiated limbs transplanted to irradiated hosts behave *herkunftsgemass* physiologically, for they regenerate though the limbs of the host are unable to do so. Such experiments demonstrate again that the source of the cells of the new limb is the stump and not the other parts of the body. An even greater localisation is possible. By shielded irradiation, regenerative capacity may be preserved in the middle part of a newt's limb while at the same time it is suppressed in the distal and proximal parts of the limb (Scheremetieva & Brunst[2]). Different tissues have different thresholds of sensitivity; thus Scheremetieva & Brunst[1], X-raying the tail of *Pelobates*, found that the regenerative potencies were lost in the following order: notochord > neural tube and musculature > gelatinous connective tissue > blood-vessels and epidermis. By careful choice of dose, it is possible to maintain limbs in normal life for indefinite periods without any capacity for regeneration (Brunst & Scheremetieva[2]).

Umanski[3] saw that the inhibition of regenerative capacity made it possible to follow up in more detail the participation of single tissue components in the regeneration process. He therefore transplanted non-irradiated limb skin to an rradiated limb, and found that after amputation normal regeneration occurred. That this was not due to a lifting of the inhibition on the internal tissues (muscle and skeleton) he proved by using skin from a white axolotl as the graft and an rradiated limb of the black axolotl as the host. The limb which regenerated was entirely free from pigment, which suggests that in this case the epidermis can tself give off cells capable of forming everything that there is in a non-irradiated regenerated limb. When skin from the head, neck or flank was used as transplant o regeneration occurred (cf. the experiment of Voronzova[2] above, p. 438); when il skin was used, the regenerate was a tail; when skin from the hind-limb region as used but not from the hind-limb itself, regeneration was very imperfect. hen non-irradiated femoral skin was transplanted to the tibial region of an radiated limb, the regenerate after amputation was a fairly normal limb (cf. the rinciple of exclusively distal regeneration, p. 433).

Umanski[4] also examined the part played by the muscles. Muscle from the dorsal surface of the axolotl was inserted into an irradiated limb in replacement f the proper limb muscles; in agreement with some of Voronzova & Krascheninikova's experiments no regeneration occurred. Even when non-irradiated dorsal muscle was combined with non-irradiated limb skin on an irradiated limb stump, no regeneration took place.

It is interesting that developing axolotl limb-buds are inhibited by the same oses as are required to inhibit their later regeneration (Brunst), and that lesser oses prevent digit formation and complete differentiation (Puckett[1]).

Summing up what has been said on this subject, we seem to see in the processes f amphibian regeneration something analogous to the induction of limb buds in normal development.* It seems likely that the competence of the rea "undetermined" mesodermal cells is rather definitely limited, and that the only undetermined in the sense that they may form any tissue within the s of the organ district where they originate. Inside these limits, however, proc go on which it is hard to call by any other name than that of induction example, the influence of the stump-skeleton on the new cartilage, the influ of the muscles on the new bone and the influence of epidermis on all the int contents of the limb.† Notable in this connection is the fact found by de Gi namely that young blastemas of limb or tail implanted on to other parts o body may sometimes avoid resorption but if so they grow without differentia Long projections are formed covered with epidermis and filled with nondes mesodermal cells, and these can even regenerate similar projections if the amputated.‡ One gains the impression that nothing is missing but the indu or inductors. Perhaps the whole process of regeneration partakes of the na of homoiogenetic induction, the mesoderm still retaining wide but defin limited competences.

If the conclusions reached above are correct, tail blastema in adult life ha limb competence but has re-acquired lens competence (see p. 296; Sch & Hummel; Schotté[6]) and perhaps ear competence (H. S. Emerson). This w amount to a reversibility of determination—a process which we have alr envisaged as taking place in ascidian life histories (p. 110). It may be desi to have a new word for this reappearance of competence. "*Recuperation* competence is suggested, and will be so used elsewhere in this book. Accor to Schotté[6], ectoderm which has lost lens competence will recuperate it if allo to regenerate over the eye-cup, and Poležaiev[4] could prolong limb competen anurans by injury (p. 439). Similarly, competence for specific differentiat stimulated by androgens is much more marked in the regenerating anal f the fish *Platypoecilus maculatus* than in the normal anal fin (Grobstein).

2·72. Biochemical aspects of regeneration

We now come to the more chemical researches. Since de-differentiation ir stump plays an essential role in preparing the competent blastema, we ha problem not entirely unlike that of insect metamorphosis (see pp. 470 ff.), and not surprising, therefore, that interest has been concentrated on autolysis the proteases generally.

Okunev[1] studied the hydrogen-ion concentration of the tissues of the generating amphibian blastema, using a Biilmann-Lund-Cullen electrode. *p*H of the normal tissues was found to be 7·2; during the period of wou healing, it fell to 6·8, rose again slightly, then fell to 6·6, a minimum at first appearance of the blastema as a noticeable bump (6 days); after whi ontinuously rose to regain its original value at 18 days after amputation. This rocess repeated itself regularly in all Okunev's experiments. A later parallel nvestigation (Okunev[2]), on a Pacific crab (*Paralithodes kamschatica*) showed hat the same acidosis could be observed during the regeneration of the claw. The normal level was here 7·0 and the minimum 6·7. Okunev[3] then studied he buffering power of the amphibian blastema, and found that it was at a naximum during the time at which the *p*H itself was at a minimum. The hange was regular and repeatable. The suggestion was made that substances of he nature of chondroitin sulphuric acid (cf. p. 10), produced by the de-differentiaion of the cartilage stump, might be responsible for both the low *p*H and the high uffering power (cf. the work of Nassonov[4] just referred to, p. 438).

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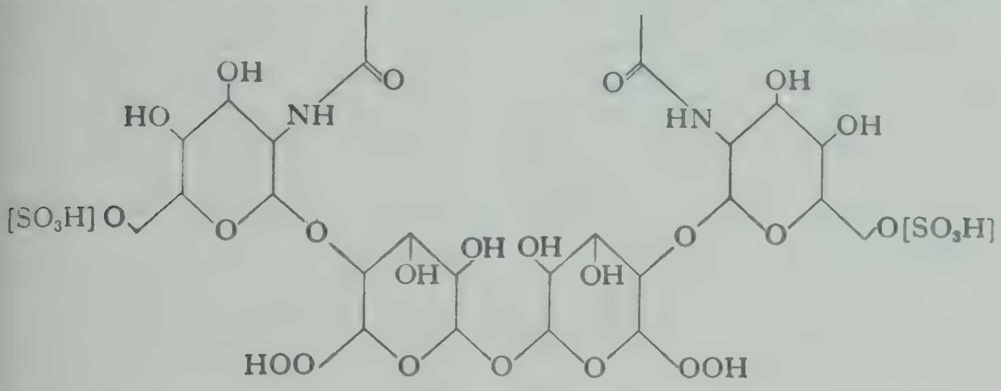
* It is surprising that no one has tried the effect of transplanting ear and nose tissu regenerating blastemas in view of the work of Balinsky discussed on p. 305.

† So also the induction of the ventral locus by the rachis in avian feather regeneration (see th mentioned on p. 303).

‡ Knowledge of what morphogenesis blastemas are capable of performing in isolation *in* would be valuable, but apparently it is not yet to be had.



Chondroitin sulphuric acid (Levene) = 2 acetyl chondrosin sulphuric acid.
Chondrosin = 1 hexosamine + 1 glycuronic acid.

Okunev[4] also investigated the oxidation-reduction potential of the blastema ssue. From the normal level of 225 mv. the E_h fell to a minimum of 180 mv. t the 10th day from amputation, after which it rose, regaining its normal value t 30 days. The values were steady and repeatable. Okunev suggested that this sult might be connected with variations in the sulphydryl content of the astema. The higher the concentration of glutathione, the lower the oxidation-duction potential might be expected to be. This hypothesis was tested by echovitch[1], who found 20 mgm. % in the normal axolotl tail but just over mgm. % in the regenerated tail from the 5th to the 10th day after amputation, ter which the concentration fell to normal. The concentration was also raised ut variable) in the stump immediately beneath the blastema. These results, in reement with the nitroprusside test as used by Coldwater[2], were not confirmed Maluf[1], who finds a slightly lower sulphydryl concentration in the regenerating il blastema (*Rana clamitans*).

	Glutathione mgm. % wet wt.
Distal tip of tail at amputation	27
After 3 weeks' regeneration:	
Regeneration blastema	18
Adjacent piece of stump	22
Proximal part of tail	30

ore work will be required on this subject, which is not without interest in view the facts described in the section on nuclear inductors (pp. 420 ff.).

The other suggestion made by Okunev[6] in order to explain the low rH of regenerating blastema was that deficiency of blood-supply brings about defic oxygenation of the cells. This explanation would not exclude the former on

Of special interest in the comparison of the blastema with embryonic neoplasmic tissue is the study of its carbohydrate metabolism. Yet as far as be ascertained little work has been carried out in this direction. Pentima examination[2] of the respiratory and glycolytic rate was limited to repair tissu mammalian muscle (he obtained a respiratory rate slightly higher than nor and a small aerobic glycolysis). For the amphibian blastema all that can be is that two investigators have found a lactic acid content considerably higher t normal; thus Okunev[5] found 18 mgm. % lactic acid in the tissues of the nor limb, and 39 mgm. % in the as yet undifferentiated limb blastema. Vladimir made a somewhat fuller study, dropping the blastemas into liquid air to av glycolytic activity during the preparation of the specimens. This will account the fact that her values were lower. In the normal limb she found 9 mgm. on the 6th day after amputation, 13 mgm. %; on the 16th, 16 mgm. % maximum), after which there was a steady return to normal. This may im a high anaerobic glycolysis analogous to that of embryonic tissues, or there exist, as in tumours, an aerobic glycolysis. We are reminded also of the cumulation of lactic acid which occurs in the hen's egg a few days a development has begun (*CE*, p. 1051). The further investigation of the car hydrate metabolism of the blastema would be well worth while.

Another piece of work of Vladimirova's[2] was the estimation of the free ami nitrogen in the blastema. As in the case of lactic acid, the figures are reason ones, showing also a rise to a maximum followed by a return to normal. A decrea amino-acid oxidation may be involved here as well as an enhanced turno connected with the formation of the new tissues. Vladimirova's work was c firmed by Orechovitch & Bromley, who obtained the following figures:

Percentage of total	Normal tail	Blastema
Non-protein nitrogen	21·6	36·2
Free amino-nitrogen	16·8	35·1

These workers also adapted the ninhydrin reaction to histochemical use, v results confirmatory of the chemical estimations.

Equally important is the state of the tissue proteases in the blastema, and th also have received attention. Bromley & Orechovitch[1] first found that the inten of autolysis in the blastema, the normal tail, and the stump, was very uneq In terms of non-protein nitrogen liberated upon 12 hr. autolysis, the figures v (*a*) for the normal tail, 58 mgm. %, (*b*) for the tail blastema, 100 mgm. %, (*c*) for the stump tissue, 233 mgm. %. The two latter showed thus a m thorough-going autolysis, indicating a higher activity of the tissue katheps When directly tested (Bromley & Orechovitch[2]), using glycerol extracts blastema and stump material, acting on normal tail as substrate, this prove hold good, though the differences were not so large. It appeared also that proportions of kathepsin bound and free differ in the blastema and the nor tail. Whereas free (lyo-) kathepsin seemed equally present, there seemed to more bound (desmo-) kathepsin in the blastema. Finally Orechovitch, Bromley & Kozmina made comparative estimations of kathepsin activity (in glycerol extracts) in the blastema at different stages of its regeneration, using gelatine as substrate.* The activity was found to reach a maximum fairly early (3 days) after amputation, while the blastema was as yet extremely small. Later it fell slowly to normal. The picture was completed by Orechovitch's estimation of sulphydryl compounds in the blastema, already mentioned[1], for as it is known that -SH increases kathepsin activity, the high concentration of it in the blastema would fit in with the increased enzyme activity. This work has been extended by Ryvkina; Ryvkina & Striganova, and Striganova. It agrees very well with the histologically observable fact that de-differentiation occurs in the young blastema.

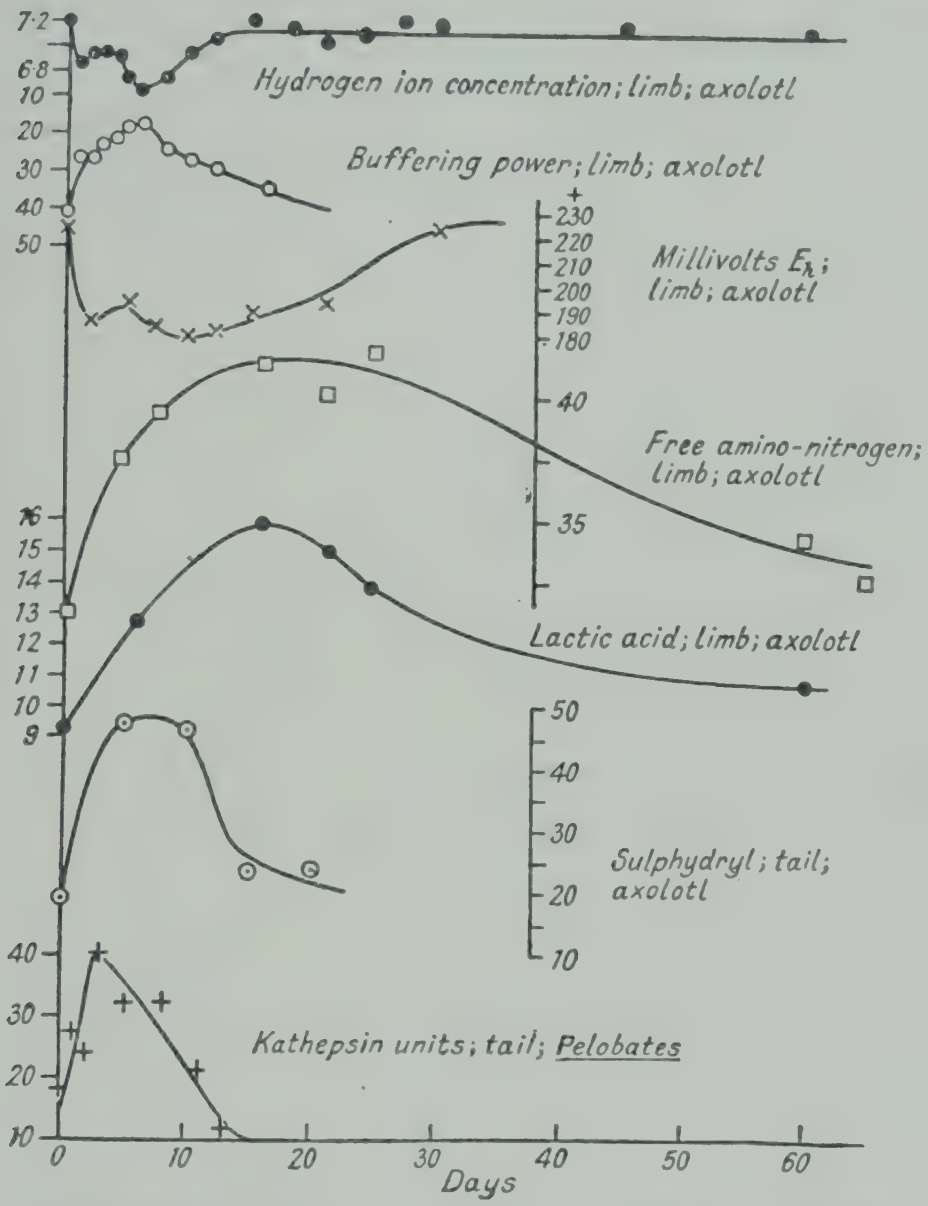


Fig. 252. Biochemical changes in the regenerating amphibian limb and tail blastema.

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* A similar piece of work was carried out by Blacher & Tschmutova, but as it involved the mitogenetic ray technique, we shall not consider it here.

Some of the quantitative relationships which have so far been mentioned a placed on the same time scale in Fig. 252, from which it can be seen that there already a good deal of biochemical regularity observable in the regenerati process. Since both limb and tail regeneration have been studied, the curves a not all exactly comparable, but they show sufficiently well the general course events.

The regeneration blastema of tail or limb seems to possess considerable hi tolysing activity. Orechovitch & Bromley covered tail blastemas with flaps skin, waiting till after the appearance of epidermis on the blastema so that t regeneration should not be inhibited (cf. p. 437). After a certain lapse of tin the flaps were seen to decay, and the regenerating member broke through. Th this was due to enzyme action and not mechanical pressure was proved l implanting small pieces of normal limbs or tails underneath back or side skin. Although the pressure was in some cases considerable, the overlying skin never gave way. This histolysing power of the blastema agrees well enough with what has already been said about its kathepsin activity and the high concentration of free amino-nitrogen in it, but it must be remembered that the enzyme may have a synthetic function, and this rather than the histolysis of stump tissues may be the meaning of its high level of activity here.

Subsequently Orechovitch[2] extended his work to the dipeptidase of the blastema. Unchanged in activity on the 1st day it rose to a maximum on the 10th and then returned quickly to normal (see Fig. 253). This behaviour of kathepsin (Orechovitch[5]) and dipeptidase was not found in the regeneration of th avian liver (Orechovitch[3]), where of course no blastema formation occurs. Kathepsin of the blastema, unlike that of normal amphibian tissue, is not muc activated by further addition of sulphydryl groups (Orechovitch[4]), and no amour of extra cystein will raise the autolytic activity (or the kathepsin activity as teste on gelatin) of normal tissues as high as that of the blastema. Mergassova the found that a boiled extract of blastema tissue will activate tissue proteases, bu the nature of the thermostable activator is as yet uncertain.

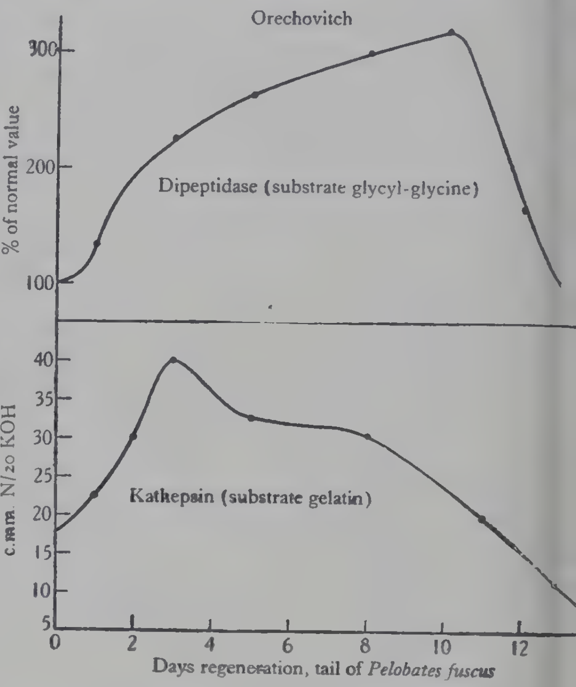

Fig. 253. Protease activities in the regenerating tail blastema.

Along this line of thought the question may be raised whether tumour tissu possesses proteases of nature or activity significantly different from normal tissue There is a considerable literature on this subject, but the best opinion is tha no such differences have yet been demonstrated (see Maschmann & Helmert).

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* But a certain rise was later found to occur in the early stages of regenerating rat liver (Orecho vitch[6]).

In sum, it must be admitted that the chemical work on regeneration is only as yet in the preliminary stages. It will not advance further until some methods are devised (such as the microchemistry of frozen disc sections, of the Copenhagen school) for studying the individual regions of the blastema, rather than the blastema as a whole.

Some of the questions with which this section opened have now been answered. Regeneration is a repetition of ontogenesis in so far as the organ districts nvolved are the same, but the processes are of necessity somewhat different. There is probably a more restricted set of competences in the reacting material, but within the limits of the organ district in question the material is certainly undetermined. Alterations of protease activity have been shown to accompany regeneration, but we cannot readily compare the biochemical changes with those of normal ontogenetic limb or tail formation since literally nothing is known about these.

2·75. Metamorphosis, competence, and the last inductors

From the standpoint of the present book the interest of amphibian metamorphosis* lies in the fact that the changes occurring during this process are due to the last of the three great inductor types, namely, the developed and unctioning glands of internal secretion. The first two of these types have already been described; the nuclear inductors derived from the genes, and the true mbryonic inductors, the evocators. The morphological changes of amphibian metamorphosis are the last stages of morphogenesis before the adult form is eached; they correspond, therefore, to the childhood and puberty stages of mammals and man when systematic changes in organ proportions and body form re going on (cf. the reviews of Davenport[2,3,4]) under the action of circulating timulators such as the growth hormone of the pituitary (cf. the reviews of Collip). All such post-natal stages come therefore under the head of "functional fferentiation". But in amphibian metamorphosis we may still trace the typical lations of inductor, competence, etc. modified in special ways, which were aracteristic of earlier embryonic periods. And the presence of the original gan districts (p. 288) betrays itself in a particularly striking manner, for eir reactions to the new circulating stimulator, when it comes, are different, nd indeed opposite.

External signs of the metamorphosis of the anuran tadpole are, of course, the owth of the limb buds and the resorption and atrophy of the tail. But there e also internal changes, such as the histolysis and far-reaching reorganisation the gut, the stomach and the pancreas. The valuable reviews of B. M. Allen[4] nd of Aleschin[2] will enable us to omit detailed descriptions of these, and, avoiding ny references to secondary problems, we shall only give an account of the essential

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I exclude from consideration here the metamorphosis of insects and other invertebrates, which s little in common with that of amphibia except the abstract statement that it *is* the transition tween the larval adaptation and the adult form. Something will be found about it, however, ove, in connection with genic effects on imaginal discs, etc. (pp. 391 ff.), and below, in conction with determination problems in insects (pp. 463 ff.). Recent work on the biochemistry of e process is discussed on pp. 470 ff.

features of present-day knowledge, regarding metamorphosis in the light of has been said above on the chain of inductors during development (p. 290

The pioneer work of Gudernatsch[1] in 1912, in which he demonstrated thyroid gland tissue fed to tadpoles accelerates metamorphosis (cf. his review[2]) was followed by the converse demonstration (Allen[1]; Hoskins & Hos that removal of the rudiment of the amphibian thyroid gland inhibits m morphosis. That iodine was the essential element was soon afterwards show Swingle[1], who was able to produce metamorphosis by the action of inorg iodine not only in normal anuran larvae but also in those from which the thy gland had been removed. For urodeles (where the changes concern mainly loss of the dorsal fin, the disappearance of the external gills and the flatte

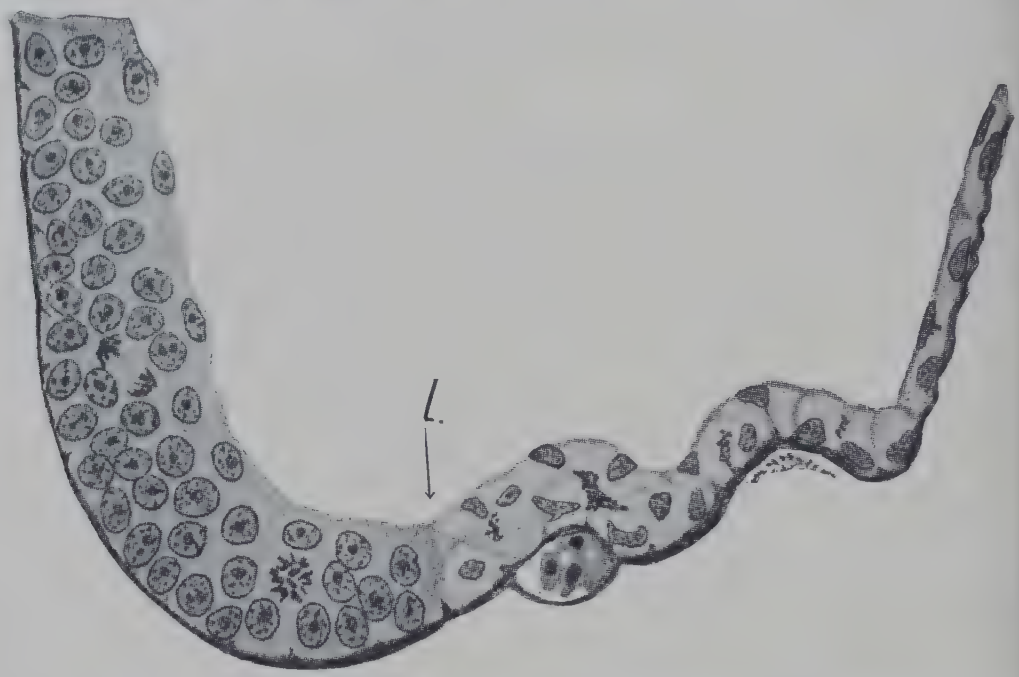

Fig. 254. Differential response of limb-bud and gill tissue to thyroxin; the line separatin two districts, one growing, the other degenerating, is very sharp.

of the body*) the same success was attained, after some difficulties, by Hu & Hogben in the case of the newt and Blacher & Belkin in the case of the axo But, as will be mentioned later, thyroxin is much more effective than inorg iodine in stimulating metamorphosis. Again, the converse experiment, th maintaining tadpoles under conditions of strict iodine starvation, was carrie by Lynn & Brambel; no metamorphosis occurred.†

The most exhaustive study of the changes in the tissues caused by appearance of thyroxin in threshold concentration in the blood circulation is of Champy[1]. He made mitotic counts in the various regions, and noted remark sharp dividing lines between the different organ districts. For example, Fig.

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* Urodele limb buds arise, of course, quite independently of thyroxin (Uhlenluth[4]). Ur balancers always disappear in accordance with the age of the donor, not the host; this pr also appears therefore to be independent of thyroxin (O. Nakamura[5]; Kollross).

† It would be interesting to study the origin and development of the thyroid in tree-frogs, the tadpole stage is normally suppressed (cf. p. 32); a beginning at this has been mac Glynn on *Eleutherodactylus*.

aken from his paper, shows a section with epidermis of the fore-limb bud on the eft and the lining of a branchial cleft on the right. The former has reacted to he thyroid hormone by growth, seen here as numerous mitoses and crowded uclei; the latter by degeneration, seen here as shrunken nuclei and vacuolation. he limit between the two zones is that between one cell and the next; there is o transitional region whatever.

Many transplantation experiments have shown that the time of metamorphic ransformation of an organ depends not on its own age but upon the onset of etamorphosis of the animal into which it has been implanted.* Tissues thus ehave invariably *ortsgemäss* with regard to the time of their metamorphic ansformations, even if they are considerably younger or older than their host.† his synchrony has been shown for the gills by Kornfeld, for the intestine by embrat, for mouth-parts by O. Nakamura[4], for the tongue by Helff[4], and for e skin by Uhlenluth[2] and Voronzova & Liosner. In Vrtelówna's interesting xperiments on *Pelobates* it was found that when eyes from younger larvae were ansplanted forwards to older ones, or *vice versa*, not only did the morphogical changes of the graft eye occur at the same time as that of the host hlenluth[1]), but physiological changes also, such as the reactivity of the is to light and to drugs, occurred simultaneously. These facts all show that mpetence to react to the stimulus of thyroxin must arise some time before e threshold concentration in the blood is attained. Otherwise younger, grafted, es would not be able to react before their presumptive metamorphic age as ey do.

In most of the above cases the grafts act purely passively. If they are taken om stages after the beginning of metamorphosis, however, they may induce etamorphosis in their younger hosts, perhaps because they contain a small nount of thyroxin (Liosner & Voronzova[4]).

On the other hand, many workers (Romeis; Jarisch; Champy[1]) have found that ore a certain age is reached the thyroid hormone has no effect on the tadpole's sues. This fixes the point of onset of competence. It is probable that the lerly succession of steps in metamorphosis (growth of hind-limbs, shortening intestine, emergence of fore-limbs, loss of chitinous beak, tail atrophy, widening mouth, etc. etc.) is due to a series of thresholds of response (Blacher; Etkin[1]; en[2]), for it is noticeable that the weaker the concentration of thyroxin the more effects produced are restricted to the earlier members of the series. At the ne time, Allen's quantitative experiments[2] showed that time of exposure to roxin is also a factor, and that given a sufficiently long time, weaker concentrans will perform as much as stronger ones. This suggests that the metamorphic anges depend on rates of different chemical reactions, catalysed by thyroxin,

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These experiments parallel those of the school of Harrison on "marking-time" and "catching-" already described (p. 347).

There is an analogy here with insect metamorphosis. Frew found that isolated imaginal discs the blowfly would invaginate when explanted into a pupal lymph medium, but would not do f the medium was made with larval lymph. Hormonic action on pre-existing competence is s a certainty here also. Analogous work on the silkworm by S. Fukuda[1] shows the same thing; al ovaries transplanted into pupae undergo a greatly accelerated development.

or for which thyroxin is in some way necessary.[*] The later phases would be those with the slowest reactions (cf. the kinetics of gene action, discusse pp. 391 ff.). It is interesting in this connection that metamorphosis will not g at temperatures below 7° (Taniguchi; Belkin) or 5° C. (Huxley[8]) though the la live unharmed. At higher temperatures, according to Tchepovetsky, the sp of metamorphosis in the presence of thyroxin is up to a certain point dire proportional to the temperature, though higher temperatures alone have effect (see also pp. 336 ff., 516).

Some light has been thrown upon the problem of competence by experim on those forms which have lost the power of metamorphosis, and which re the stage of sexual maturity while still retaining the "larval" characteri (neoteny). The evolutionary significance of this fact has been discussed by de Be Here we are interested in the mechanism of neoteny. With the classical exan before them of the Colorado axolotl,[†] *Amblystoma tigrinum*, which may assu either the neotenic or the metamorphosing state, and in which metamorph may be induced by iodine (Hirschler; Blacher & Belkin), a number of wor tried to induce metamorphosis in the perennibranchiate, *Necturus*, but with success.[‡] When investigations of the hormone content of the glands of animal were made, it was found that the thyroid gland contains active thyro (Swingle[2]) and the pituitary contains active pituitrin[‖] (Charipper & Corey); cf. role of the pituitary gland (p. 454 below). The inhibition must therefore in the tissues; a loss of competence, not a loss of inductor. This case wo therefore clearly join the facts known about the presence of embryonic evoca in adult tissues (p. 172) and the loss of balancer competence in the axolotl the retention of the balancer inductor (p. 342). But we have also to take account certain substantial differences between the blood-vessel systems in axolotl and *Necturus* (Figge[1]; Gilmore & Figge), such as the absence of the s branchial arch in the latter. It is thought that the attainment of an effic pulmonary circulation in perennibranchiates has been rendered difficult by disappearance of a specific anatomical structure. At the same time, the c petence of the external gills for reacting to the thyroid stimulus by atrophy certainly disappeared. Figge[2] was afterwards able to show that ligating the s branchial arch in the axolotl delays, though it may not entirely prevent, m morphosis induced by thyroxin injections, especially if the temperature is so that the metamorphic tendency is small. Yet in depulmonated axolotls m orphosis is still possible, cutaneous blood-vessels taking on an enhanced respira- ory function (Garber; Koschtojanz & Mitropolitanskaia).

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[*] It has been found, too, by Voronzova & Liosner and others that, after a certain stage has reached, metamorphosis will go to completion without further additions of thyroxin. This sug that the mechanism might be that of a co-enzyme. Skin from an axolotl half-way through m morphosis, transplanted to an axolotl receiving no thyroid, will complete its metamorphic cha in the new situation.

[†] The axolotl's metamorphosis, first reported by v. Chauvin in 1885, has a review all to it that of L. Marx.

[‡] Except for the uncertain effects which Gutman was able to produce by the simultaneous of thyroxin and adrenalin.

[§] Active because when implanted into frog tadpoles metamorphosis was induced.

[‖] Active because when the anterior lobes were implanted into frog tadpoles metamorphosis accelerated (thyreotrophic hormone).

The effect of thyroxin has also been tried on other vertebrates.* It seems that Veiss[8] was able to accelerate tunicate (prochordate) metamorphosis by thyroxin hough Bradway could not, and according to Baumann & Pfister dilute thyroxin olutions cause the disappearance of aortic arches in trout embryos. On the other and, Horton found no effect of thyroxin on the ammocoete → lamprey meta- orphosis. The ammocoete thyroid gland contains no iodine and cannot produce etamorphosis in the frog tadpole, though that of the lamprey can.

We come now to the most striking regressive change at metamorphosis in mphibia, namely the resorption and atrophy of the tail. It was originally thought y Barfurth[1] in 1887 that the growing urostyle (the unsegmented posterior portion f the vertebral column) occluded the caudal blood-vessels so that atrophy ollowed death by asphyxia. This view was accepted as late as 1922 by Bradley[2] nd W. Morse, who were the first to regard the tail resorption as an autolysis. ut Helff[5] a few years later decisively disproved it by extirpating the urostyle efore metamorphosis, after which tail resorption proceeded quite normally. e also showed that ligation of the aorta gave no tail atrophy (Helff[1]), but at injection of small amounts of lactic or butyric acids would do so, autolysis eginning at the site of injection and spreading over the whole tail.

Helff's conclusion was clinched by the demonstration of Helff & Clausen that nuran tails transplanted to the back underwent there the typical atrophy of etamorphosis. The converse of this experiment was made by Lindeman, who ansplanted body skin to the tail before metamorphosis, with the result that no trophy of this particular epidermis took place. Competence to atrophy in sponse to thyroxin therefore not only appears at a definite time but also only definite places. Brilliant establishment of this principle came from the work f Schubert, who transplanted limb buds to the tail, and still more from that of hwind, who transplanted eye-cups there. As in Fig. 255 (opp. p. 464) the eye oved gradually forwards during tail resorption, and finally came to rest in the ral region at the conclusion of metamorphosis. Clausen, transplanting skin and uscle from different regions of the tail to the back, found that those from the terior part atrophied more rapidly and completely than those from the posterior rt; a finding in agreement with Dunihue's experiments on rate of autolysis *vitro* at pH 6·6 of parts from anterior and posterior regions. Another interesting periment was that of O. Nakamura[2], who exchanged, at early tail-bud stages, uran (*Rana*) with urodele (*Hynobius*) tails. Anuran tails on urodele bodies rophied completely at metamorphosis; urodele tails on anuran bodies were st covered with anuran epidermis and this then atrophied at metamorphosis, aving the urodele tissues naked.

Including the chick embryo (injections into the egg with little result, Guelin-Schedrina[1]). The yroid of the chick embryo, however, can respond to the thyreotrophic hormone of the pituitary tuditsky; Woodside[2]). There is a large literature on the development of the thyroid gland and hormone in birds and mammals, which can be approached through the papers of Daineko[1]; tin; M. L. Hopkins; Aleschin[3]; Artemov & Valedinskaia; Lelkes; Marza & Blinov. On the relation of thyroid activity in embryonic life with that of other glands, see M. Aron's review.

If tail resorption is an autolysis, an acidosis, at least local, should be expe The earlier attempts to demonstrate this were not very convincing, but Alesc work[1], which gave a *p*H of 7·15 for the normal tissues and 6·65 for the tail du resorption, may be accepted. The acidity was perceptible some time befor first sign of morphological change. Parallel work by Helff[9] on the blood *p*H parallel results, as the following figures show:

	*p*H
Normal pre-metamorphosis	7·50
Intermediate stage I	7·39
Intermediate stage II	7·27
End of metamorphosis	7·20
Three weeks later	7·18
Adult (Kamm; L. H. Hertwig)	7·4–7·6

The acidosis seems therefore to be general, though it may be more extreme loc We do not yet know whether the tissue reaction of the degenerating gills* is more acid than normally. It is to be noted that the period of maximal resorp rate of the tail follows immediately after the period of maximal growth rate o fore- and hind-limbs (Champy[1]; Irichimovitch & Lektorsky; Voitkevitch[2]) Fig. 256. Any suggestion that the material of the former is necessarily use the construction of the latter has to reckon, moreover, with the measuremen O. Nakamura[3], who found that limb growth in anuran larvae proceeds just as after the tail is removed as normally. During metamorphosis the water-con of the whole body falls slightly (from 92·8 to 89·4 %), as does that of the (from 93·8 to 89·6 %), while that of the limb-buds rises (from 75·0 to 86·4 a fact contrary to the association between high water-content and pluripote but probably connected with the changing proportions of the skeletal stituents (Irichimovitch[2]). These changes proceed in urodeles, where the ta retained, just as in anurans, where it is lost. From the interesting weighing Voitkevitch[2] (see Fig. 256) we see not only that tail resorption follows upon maximum growth period of the hind-limb, but also that gut reorganisation so, and that both these processes are immediately preceded by a spurt of gro in the thyroid gland itself. The data from Champy[1] show at the same time in thyroid-accelerated metamorphosis the same precedence of limb-bud gro over tail resorption is to be seen.

The nitrogen metabolism might well be expected to be affected by tail res tion. Van der Heyde was unsuccessful in finding a corresponding rise in nitrogen excreted, but this was observed by Blacher & Efimov.† Liosner & Bla found an 82 % increase of free amino-nitrogen in the tail tissues preceding morphological changes, and later an increase in polypeptide nitrogen. In atrophic gills there was a 45 % rise in free amino-nitrogen. Though the nitrogen of the blood was raised at the beginning of metamorphosis (by pro substances), the free amino-nitrogen was constant, later decreasing. Substa changes in the activities of enzymes in the gut (Blacher & Liosner; V. Doljan and in the stomach (Liosner[1]) during their reorganisation have been obser The proteases of the blood may also be increased during metamorph according to von Falkenhausen, Fuchs & Schubert, but this requires confirmat

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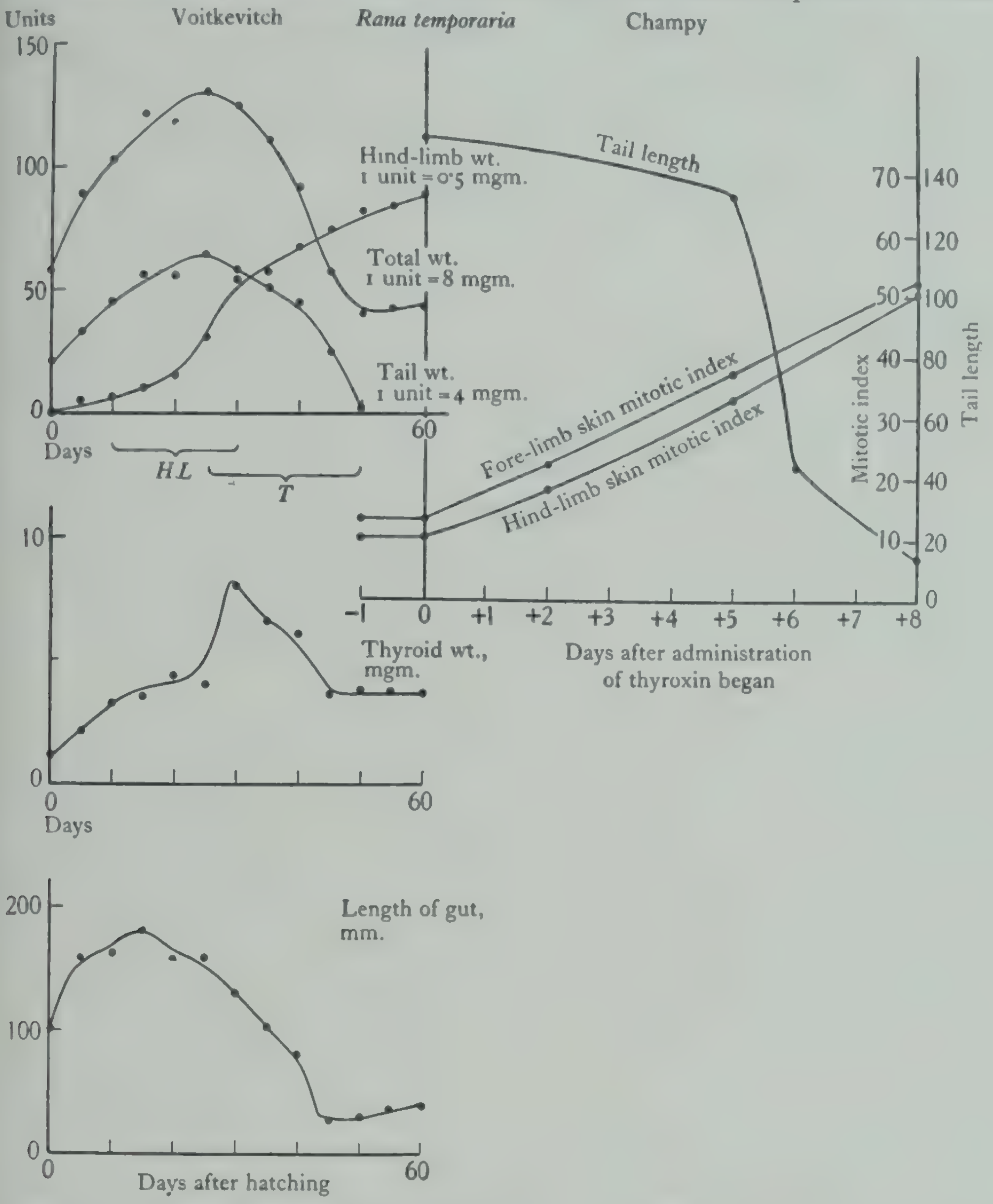
* Cf. the interesting histological account by Grotans[1].

† See also p.

On the whole the metabolic work so far done tells us little regarding the nature f the characteristic competences for atrophy of tail and gills, but a new attack the light of modern knowledge could readily be made. It is still uncertain what extent leucocytic infiltration plays a part in tail resorption. A dissociation



. 256. Temporal relationships of organ changes in amphibian metamorphosis. *HL*, Hind-limb maximal growth-rate period; *T*, tail maximal absorption-rate period.

tween regression and development has been worked out by Puckett[2], whose efully graded X-ray doses favoured resorption of tail and gills but inhibited differentiation of the limbs.

What effects does the thyroid hormone have on the oxidations of the meta-orphosing tadpole? In connection with another problem, that of biological ecificity, we have already had occasion to mention this point (p. 264). Groebbels[1] s the first to show that oxygen consumption of the larva rises both absolutely d relatively on metamorphosis, whether natural or provoked by thyroxin, and s has since been fully established (Abelin & Scheinfinkel; Huxley[5]; Helff[2];

Schwartzbach & Uhlenluth; Wills; the sole dissentient voice is Etkin's[2]). dinitrophenols, which stimulate metabolic rate (cf. p. 570), have no effe amphibian metamorphosis (Cutting & Tainter). Conversely, acetyl-thyr which has no effect on metabolic rate, still stimulates metamorphosis (Ke Swingle, Helff & Zwemer), whether in the mono- or the di- form (Tita Summ). It is therefore certain that the rise in metabolism which takes pla the tadpole is not a cause of, or a necessary condition for, metamorphosis.

In this connection there are some observations with metabolic inhib Hoffman found that methyl-cyanide (acetonitrile) inhibits thyroxin acceler of metamorphosis, and Demuth[1] confirmed this for KCN. Cyanide and acetate, according to him, inhibit metamorphosis but not growth, while flu inhibits both.

As has already been mentioned, inorganic iodine will provoke amph metamorphosis even in thyroidectomised tadpoles, either because other ti besides the thyroid gland can make small quantities of thyroxin from it, or be the organic part of the thyroxin molecule is only a carrier for the active ele But iodine in thyroxin is enormously more potent than iodine in other for combination (Allen & Lein). It is 300 times more active than the same am of iodine as di-iodo-tyrosine (see the formulae on p. 256) first shown effective by M. Morse, and this in turn is many times more active than inor salts of iodine.

The histological changes which occur in the larval thyroid gland have the subject of many papers (cited in Allen's review[4]), which all agree in descr a marked secretory phase during metamorphosis. That the thyroid gland its turn dependent upon the pituitary gland for a lead is now fully establi L. Adler in 1914 was the first to prove that hypophysectomy prevents meta phosis, and we know that the thyroid glands of hypophysectomised tadpoles be restored to normal function by injection of pituitary extracts (P. E. Smi I. P. Smith; Spaul). In the absence of the anterior pituitary secretion (thyreotro hormone, cf. Collip; Collip & Anderson) the thyroid gland cannot function metamorphosis cannot take place. In the absence of the thyroid gland, the thy trophic hormone of the pituitary by itself cannot induce metamorphosis (Fig Uhlenluth). But by transplanting the anterior lobe of the pituitary from tad of different ages backwards and forwards in time into tadpoles of a standard metamorphic age, Allen[3] and, later, Etkin[3], were able to show that only those later stages would induce metamorphosis. Pituitaries from younger stages w not do so.* Hence the initial lead in metamorphosis is given by the pituitary. upon what "alarm-clock" the pituitary itself depends is as yet quite unkno

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* Moreover, Etkin & Huth could obtain precocious activation of the thyroid gland by planting it to a site near the pituitary. Hence a "field" of thyreotrophic hormone m envisaged (cf. pp. 312, 418, regarding inductors of varying diffusibility).

† By taking advantage of the unequal distribution of basophile and eosinophile cells in pituitaries, Voitkevitch[3] was able to identify in amphibian experiments the thyreotrophi gonadotrophic hormones with the former and the growth hormone with the latter. Sinc thyreotrophic hormone comes from the anterior lobe, its origin might be referred back inductive stimulus from the brain whereby the gland is first formed (see p. 302). In this wa hormone could be linked in with the chain of inductors (cf. Kleinholz; Chen, Oldham & Gei

Another interesting experiment was that of Bytinski-Salz[2], who exchanged luring early embryonic life the presumptive pituitary regions of the axolotl *mblystoma mexicanum*, which never normally metamorphoses, and a metamor-hosing race of *Amblystoma tigrinum*. No effects on the growth or metamorphosis f the former were found but the latter responded, showing that the pituitary of he non-metamorphosing form contains the inductor, but that its tissues are ot competent to react to it even when provided from pituitary tissue known o be effective. But the experiments were not entirely conclusive, for it was bservable that in many cases the animals later developed the colour characteristic f hypophysectomised larvae; these on dissection showed that the transplant had lisappeared.*

Allen[4] well says that the endocrine glands "take their place alongside the genes, he physiological gradients, and the organiser as an important part of the chain f influences that control development". In the succeeding section we shall see ow true this is of insect development, wide though its divergences are from the henomena in vertebrates which we have been studying in the foregoing pages.

·8. Determination in insect development and metamorphosis

With this section we leave the domain of vertebrate experimental morphology nd come to the invertebrates. As has already been pointed out in the discussion n mosaic and regulation eggs (p. 131), many eggs of invertebrates show mosaic evelopment. Although this may be by no means so absolute as was formerly hought, it is sufficiently striking to have held back considerably our knowledge f the determination processes in invertebrates. Only in certain cases, such as e typically regulation eggs of echinoderms, which will be the subject of the ollowing section, and those of some insects (for among insects all grades of egulation are to be found), has substantial progress been made. Unfortunately p to the present no biochemical work has been done on the problems of deter-nation in insects, but the processes there are so peculiar, and parallel in so eresting a way those which we have seen at work in vertebrate development, at they merit a careful treatment.

Insect embryology has been the subject of many investigations. Those which e relevant but which for reasons of space cannot be referred to here will be nd in Seidel's accounts[6,7] of the work of his school, and in the admirably lucid iew of Richards & Miller. The development of an insect is so different from at of an amphibian that a short description of it must first be given.

The yolk of insect eggs is not concentrated at one pole as in the eggs of verte-ates, but occupies a central position (hence the term "centrolecithal eggs") where is enmeshed in a slight cytoplasmic reticulum and surrounded by a thin layer of ripheral cytoplasm. Cleavage follows the arthropod type; there is a dissociation etween nuclear and cytoplasmic division, so that a large number of nuclear divi-ons occur first, till more than two hundred nuclei have been formed, and then the ll-divisions begin to form radially when the nuclei have migrated into the peri-eral cytoplasm. Normally no development will follow if the early cleavage nuclei

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It is interesting that the water-regulatory action of the posterior pituitary does not begin till tamorphosis (Howes).

are killed (Reith[1]; Pauli), but in one very exceptional case, reported by Seiler, c divisions took place in the absence of any nuclear divisions in hybrids of moth *Phragmatobia* (cf. cell-division without nuclear division in echinoder p. 510, and later, under Dissociability). Insect development is thus fundament dependent upon the proper migration of the cleavage nuclei, and as we shall later, their presence or that of their products in a certain region of the eg essential for all further development. Many investigations have established fact that the early nuclear divisions are, as in other organisms, equipotent Thus neither abnormal nor delayed nuclear distribution, nor even the eliminat of one of the first two nuclei, will prevent normal development (Seidel[4]). At f synchronous, the nuclear divisions lose step after some time, usually when t begin to enter the peripheral cytoplasm.

The mechanism of the migration of the nuclei remains very obscure, Eastham[1], who studied the development of the butterfly *Pieris*, speaks of cen fugal streaming. The nuclei, both during and between mitoses, exist in min protoplasmic islands, with comet-like tails stretching backwards in the direct of migration. Other observers quoted by Richards & Miller give similar accou At present we are far from any understanding of protoplasmic motion. One only to read the description of the streaming and churning of the protoplasm the egg of a polychaete worm, *Sabellaria*, filmed by J. E. Harris, for examp to realise the extent of our ignorance of these processes. When to this we a that special nuclei, the vitellophages, no doubt important for yolk absorpti either remain behind in the yolk when most of the other nuclei have entered peripheral cytoplasm, or else subsequently migrate from the blastoderm into yolk (Sehl), we appreciate further the complexities of the situation. Fortunat it has been possible, taking the mechanism of these early stages for granted, find out a good deal about the determination process in those cases where occurs later.

We have already had occasion (p. 218) to mention the long-known generali tion of Hallez that the polarity of the three embryonic axes is determined alre in the ovary before laying. The egg is like a crystal with the same lattice as mother. This holds good generally for insects but not universally, for in the b *Pyrrhochoris* Seidel[1] found that the longitudinal embryonic axis may vary fr being coincident with the longitudinal egg axis to being transverse to it. insects such as the grasshopper *Melanoplus*, moreover, the embryo begins develop in the reverse position, with the head towards the posterior end, wh the micropyle is, and attains its normal position later by the active movem known as "blastokinesis" (Slifer[3]). We shall return to this point (p. 463).

2·81. Organiser phenomena in insects

Insects show all variations between mosaic and regulation eggs. The work Seidel's school[2] has suggested the following series:

Regulative Incompletely regulative M
Odonata—Hemiptera—Orthoptera—Coleoptera—Hymenoptera—Neuroptera—Lepidoptera—D

This must, however, be regarded as somewhat tentative, for practically nothi is known experimentally about the Hemiptera. Moreover, only a single spec of Odonata has been studied, two Orthoptera, two higher Hymenoptera, Neuropteron, five Coleoptera, one Lepidopteron, and three higher Dipte Possibly considerable variation within the groups may be expected, particula since the parasitic polyembryonic Hymenoptera (cf. p. 228), which have not so far been studied experimentally, would be expected to be highly regulative. The highly regulative eggs (e.g. the dragon-fly *Platycnemis* (Seidel[3]) and the camel-cricket *Tachycines* (Krause[1])) already possess at fertilisation an irrevocable antero-posterior and dorso-ventral determination. But shortening the longitudinal axis produces perfectly normal dwarf embryos, and rudiments split by injuries easily produce duplications of any organ. This may go so far as to produce one dwarf embryo almost inside another (Seidel[2]) if yolk-fissures are made by bending the egg at the beginning of development. In the incompletely regulative eggs (e.g. the honey-bee *Apis* (Schnetter), the ant *Camponotus* (Reith[2]), the beetle *Bruchus* (Brauer & Taylor), the moth *Ephestia* (Maschlanka) and the alder-fly *Sialis* (du Bois)) power of regulation is lost much sooner than in the previous group, at or shortly after blastoderm formation. In the mosaic eggs (e.g. the beetles *Calligrapha* and *Leptinotarsa* (Hegner); the blowfly *Calliphora* (Pauli); the fruit-fly *Drosophila* (Sonnenblick; Howland & Sonnenblick; Howland & Child); or the beetle *Agelastica alni* (Smreczyński)) injuries always result in losses of the corresponding parts, and all development from fertilisation onwards is self-differentiation.*

To give reality to what has been said, the illustrations of Fig. 257 (taken from Seidel[6]) should be examined. The stage of nuclear divisions is followed by the migration of the nuclei into the peripheral cytoplasm and the formation of two areas of especially intense cleavage which shortly join to form the embryonic blastoderm. The future embryonic axis is then sketched out; it bears in the centre the median plate which later will sink in forming the neural groove and ultimately the chain of ganglia in a way not unlike the formation of the central nervous system in vertebrates, save that this of course is on the ventral, not the dorsal, surface of the embryo. The first sign of segmentation (analogous with the somitic segmentation but of course to be pushed much farther) appears as a groove etween the second maxillary and the first thoracic segments. The embryo is ow in two layers. At a later stage the appendages become visible and invagina-ons at the anterior and posterior ends of the body form the stomodaeum and roctodaeum respectively. The endoderm is formed at this stage, growing back-ards from the tip of the stomodaeal and proctodaeal invaginations.† At last the rm of the completed insect or larva appears.

The basic plan of primary determination in insect eggs was revealed by eidel's classical work[3] on the dragon-fly *Platycnemis*. He found that when auterisations or constrictions of the egg with a knot of child's fine hair were pplied, what happened afterwards depended on the exact time at which they were nade. This result is shown in Fig. 258. A constriction applied in an early stage

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It is rather unfortunate that *Drosophila*, the classical object of genetic research, should happen o have a mosaic type of development. A special monograph, that of Poulson[2], has been devoted o its descriptive embryology.

The above short description does little justice to the highly complicated nature of the formation f the germ-layers in insects; still an obscure subject, as the reviews of Eastham[2] and of Roonwal[2, 3] d the book of Johannsen & Butt show. We have noted already (p. 367) clear cases of genetic iterference with normal germ-layer formation in *Drosophila*.

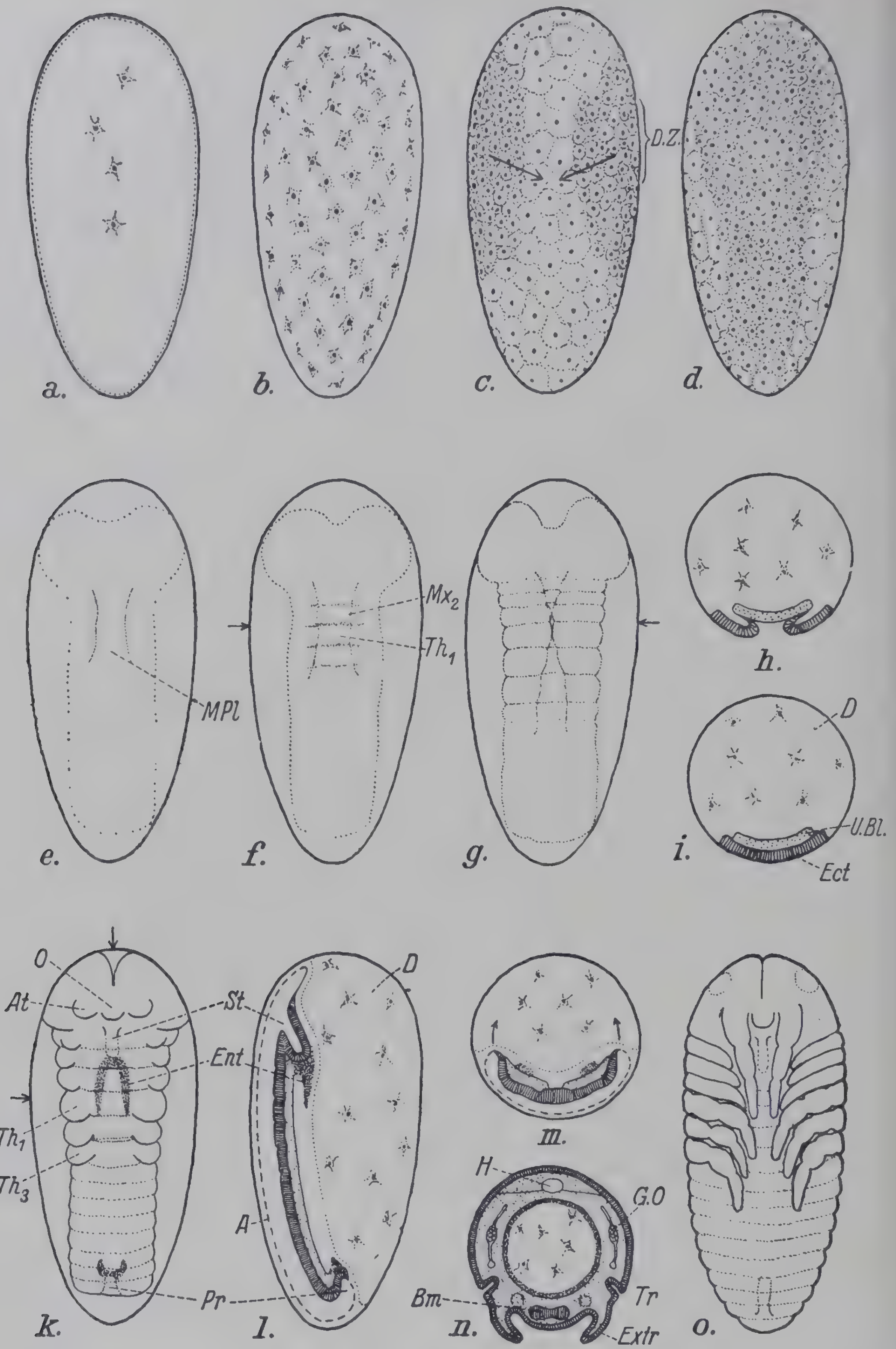
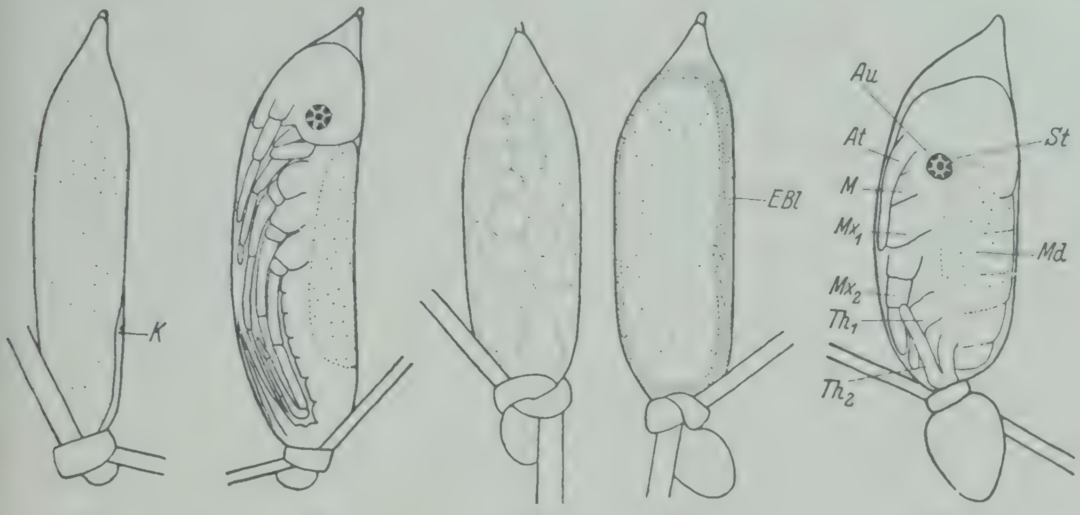


Fig. 257. Development of a typical insect egg. *a*, 4-nucleus stage; *b*, 128-nucleus stage; *c*, *d*, mation of blastoderm; *e*, formation of embryonic axis with head-folds; *f*, beginning segmentation; *g*, continuation of segmentation forwards and backwards from differentiati centre; *h*, cross-section at arrow in *f*; *i*, cross-section at arrow in *g*; *k*, appearance of org and limb-rudiments, starting from 2nd maxillary (Mx_2) and 1st thoracic segment (*T* *l*, longitudinal section of *k*; *m*, cross-section at arrow in *k*; *n*, cross-section of *o*; *o*, comple organogenesis.

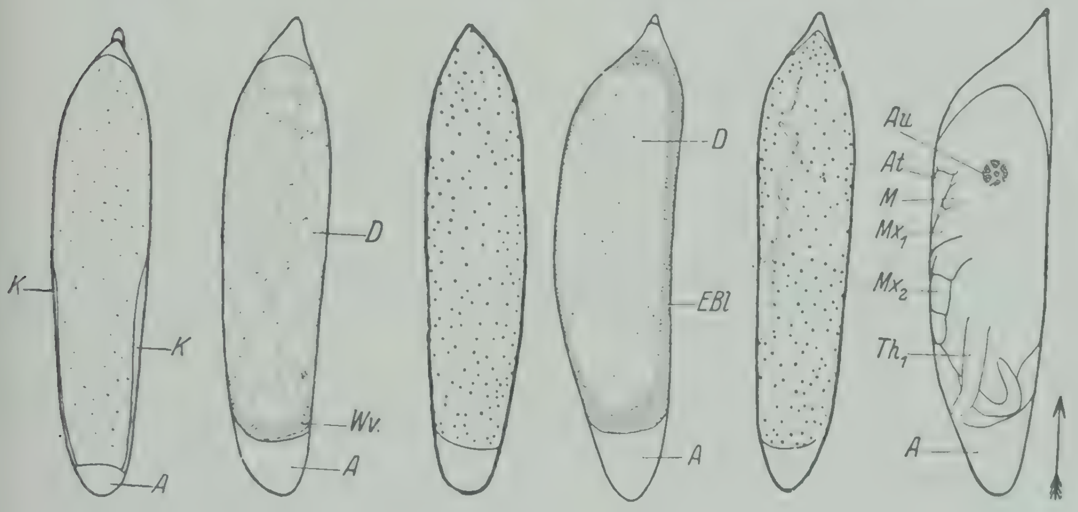
DZ, Differentiation-centre; *MPl*, middle plate; *D*, yolk; *UBl*, under plate; *Ect*, ectoder *St*, stomodaeum; *Ent*, endoderm; *Pr*, proctodaeum; *A*, amnion; *O*, upper lip; *At*, anten *H*, heart; *Bm*, ventral neural cord; *Extr*, appendages; *Tr*, trachea; *GO*, gonad.

right at the posterior end (*a*) had no effect and normal development followed (*b*). But if it was applied farther forwards, development was subsequently entirely inhibited (*c*). If, however, it was applied at the same forward point some time after the formation of the blastoderm (*d*), again normal development followed (*e*). Parallel



(*a*) (*b*) (*c*) (*d*) (*e*)

Fig. 258. Effects of constriction on a regulative insect egg (see text).



(*a*) (*b*) (*c*) (*d*) (*e*) (*f*)

Fig. 259. Effects of cauterisation on a regulative insect egg (see text).

(*a*), (*b*) Increasing extent of cauterisation; (*c*) surface view of (*b*); (*d*), (*e*) only the extra-embryonic blastoderm develops; (*f*) normal development when cauterisation is late.

Key for Figs. 258 and 259. *K*, Blastoderm; *At*, antenna; *Au*, eye; *D*, yolk; *EBl*, extra-embryonic blastoderm; *M*, mandible; *Md*, midgut; *Mx*, maxilla; *Th*, thoracic leg; *St*, stomodaeum; *A*, cauterised portion; *Wv*, wound healed.

esults were obtained by destroying the posterior regions of the egg with cautery; f this was done early, development was inhibited, if it was done later, developnent was not inhibited (Fig. 259). The time effect, graphically plotted, gave triking regularities; thus Fig. 260 shows how as time went on the cauterisation ad to be pushed farther and farther forwards in order to obtain inhibition of evelopment; cf. the arrow in Fig. 259 (*f*).

These and other facts led to the conceptions of the *Bildungszentrum* ar the *Differenzierungszentrum*, well translated respectively by Richards & Mill as "Activation centre" and "Differentiation centre". The existence of the centres has been demonstrated* in a dragon-fly (*Platycnemis*) by Seidel[3], tv ants (*Camponotus* and *Lasius*) by Reith[2], two beetles (*Bruchus* and *Sitona*) l Brauer & Taylor and Reith[3], and in the mealworm (*Tenebrio*) by Ewest. Th must be very general, probably as general as the single organisation centre is amphibia and other vertebrates. In *Platycnemis* the anterior border of tl activation centre coincides with the presumptive posterior end of the embry how far back it extends posteriorly is unknown. Its action depends upon tl arrival of the cleavage nuclei, and some consequent reaction between them ar the material of the centre. Thus if the arrival of the cleavage nuclei is delay

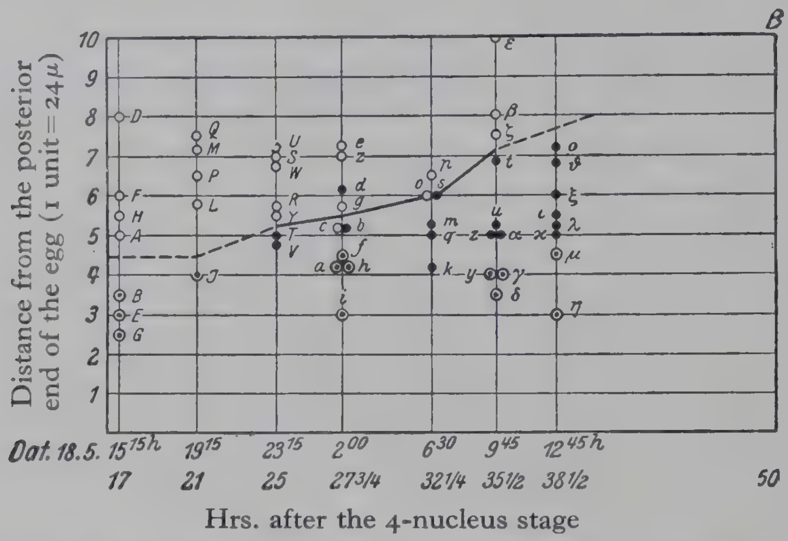


Fig. 260. Progressive change in the position of cautery required to inhibit the development a regulative insect egg (see text). ○, Inhibition of development; ⊙, normal developmen ●, formation of blastoderm; ⊙, head defects.

(as the result of killing one of the first two nuclei or constricting the egg and late removing the constriction) the function of the centre is delayed. If, howeve the nuclei are absolutely prevented from reaching the centre, no axiation an development ever follow, although an embryonic blastoderm is formed (Fig. 261 It is as if the canvas is ready, but the painter does not arrive (cf. anidian embryo of vertebrates, referred to on p. 223). Partial constrictions prove that the presenc of nuclei is essential in *Platycnemis*, but in the ant *Camponotus* Reith[2] has show that the nuclei are not essential, and that some diffusible substance precedin them is involved. In the two beetles Brauer & Taylor report that nuclei them selves are necessary. It is probably not unsafe to conclude that in some of thes cases the active substance, whatever it is, can diffuse out of the nuclei and a over the egg, while in other cases it cannot do so, or not in sufficient quantit until at the activation centre some releasing change occurs in the permeabilit of the nuclear membranes. We are strongly reminded of what has already bee said (p. 418) regarding nuclear inductors.

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* See the review of Krause[2].

Without the activity of the activation centre there will be no axiation and no development. But what precisely does this centre do? From the constriction and cautery experiments already described Seidel[5] concluded that a new substance, essential for development, was produced and diffused forwards towards the point where differentiation first visibly begins. The curve in Fig. 261 traces, indeed, the very progress of this diffusion. The most remarkable thing is that in some eggs this diffusion can actually be followed, for along with it there goes "a structural change in the yolk, which, with the loss of the finer yolk-particles, becomes more solid and more transparent" (Seidel[7], p. 415). Nothing is known about the significance of this colloidal change, but when it reaches the point where visible differentiation will shortly afterwards begin, the differentiation centre, an actual contraction of the yolk occurs. This spread of visible differentiation from the presumptive prothorax is a very general phenomenon in insects

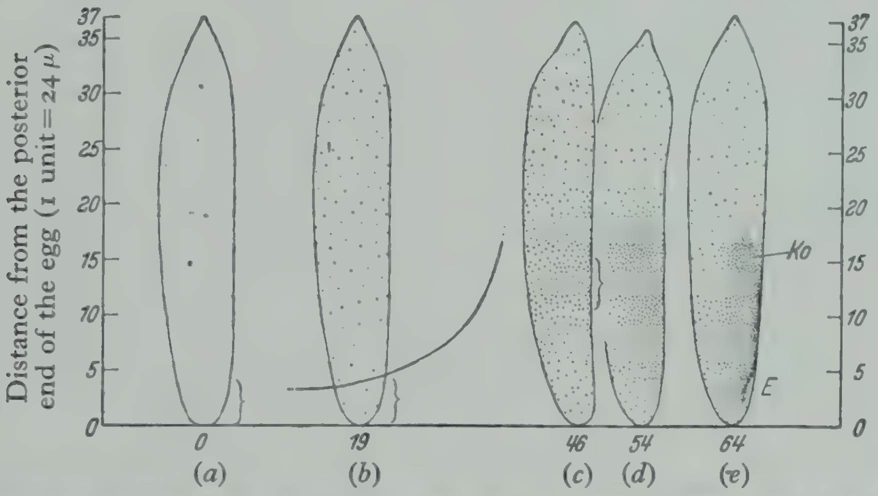

Fig. 261. Diagram showing the part played by the activation centre and the differentiation centre in the development of a regulative insect egg. (*a*) Activation centre not yet activated; (*b*) beginning of its function; (*c*) differentiation centre; (*d*) blastoderm arising; (*e*) completion of blastoderm. *Ko*, headfolds.

Schnetter), so the differentiation centre is probably very general also. In the osaic types it would presumably be stimulated at or before fertilisation. In the oney-bee, an incompletely regulative type, Schnetter found staining differences uring cleavage with dyes such as thionin (cf. above, pp. 133 ff.). Unless the fferentiation centre is present uninjured, no differentiation will take place, but efore the product of the activation centre has reached it, it is inert. The direct ction of the differentiation centre appears at least to involve a wave of contraction the yolk spreading from it anteriorly and posteriorly, the yolk contracting from e chorion in such a way as to force the sheet of blastoderm cells to fill the vacant pace. The work of Krause[1] on the camel-cricket and of Schnetter on the honeyee confirms in general this picture and suggests that the differentiation centre s concerned in determining the boundaries of the various organ regions which t this time shift out of the centre in a stream backward and forward to their efinitive positions. But it must be remembered that our knowledge of the determination process in insects is greatly hampered by the lack of the possibility of ansplanting pieces at different stages in *herkunftsgemäss-ortsgemäss* experiments.

Since the establishment of the existence of the two centres in the insect
there has been much discussion regarding their relation to the amphibian orga
tion centre. That the insect axis should form on the ventral surface and
amphibian one on the dorsal surface is a similarity, not a difference, since axia
begins on that surface of the embryo which will form the central nervous sys
Weiss[16] has made the suggestion that the "activating and organising functio
which in amphibian development are combined in one centre are in the ins
separated into two centres. From the context, however, it is probable tha
had in mind the distinction made in this book between evocation and indivi
tion, i.e. the chemical stimulus to neural determination and differentiatio
the one hand, and the forces, probably of field character, which govern
regional differentiation of the evocated axis. This distinction will not cove
facts of insect development.* On the contrary Richards & Miller's transla
of *Bildungszentrum* as activation centre is especially happy, because it is b
to regard what happens in the activation centre as analogous to the setting
(activation) of the amphibian primary evocator from its inactive combina
(see p. 170). Then instead of having to travel only the very short dista
separating its site of origin in the archenteron roof from its site of reactio
the dorsal ectoderm, it has to travel the greater part of the length of the in
egg. These considerations may be made clearer in the form of a table:

		Cause	Effect
Amphibia	Beginning of gastrulation	Change of metabolism	Liberation (activation) evocator
	Later gastrulation	Evocator	Neural determination differentiation
Insects	Activation centre	Arrival of nuclei or substance produced by nuclei	Formation of chemical stimulating substance
	Differentiation centre	Arrival of chemical stimulating substance	Contraction of yolk m fibrils, and determin processes

On this scheme the product of the activation centre would logically corresp
to the primary evocator. At the same time it invites the thought that in amph
also the local change in metabolism which occurs at the beginning of gastrula
may be conditioned by chemical products of the nuclei, and as we have alre
seen (p. 203), the work of Brachet[18] on the fixed –SH distribution is poin
fairly clearly in this direction. It should furthermore be possible to reve
metabolic change in the activation centre when the migrating nuclei or t
product arrive in it. It should also perhaps be possible to activate the differen
tion centre of an insect egg by chemical preparations of the activation cen
One would like to know whether denaturing the proteins, for example, of
activation centre, would liberate any substance capable of stimulating
differentiation centre, without any nuclei or their products ever having b
involved.

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* Spemann's suggestion (made in ch. 18, which was omitted from the English editio
Embryonic Development and Induction) was less helpful; he compared Seidel's two centres t
two determinations of double assurance. But the necessity for this concept is now doubtful
pp. 293, 308).

From what has so far been said it is clear that actual contractile processes of he yolk micro-fibrils (cf. p. 674) play a greater part in insect development han in that of other forms. We are reminded of the curious contractions of the lastoderm in fishes already mentioned (p. 74). And in the later development f some insects, the role of contraction processes is still further notable. As has lready been mentioned, in the grasshoppers, embryonic development begins vith the cephalic end of the embryo at the posterior end of the egg, but at a elatively later stage, when the length of the embryo is just less than half that f the egg, "blastokinesis" (sometimes called "revolution")* occurs, a process y which the embryo moves round until its head is at the anterior pole of the egg. rom the description of Slifer[3], this process, which does not begin to occur until good deal of liquefaction of the egg-contents has taken place due to imbibition f water (see p. 37), is carried out by waves of contraction originating posteriorly nd running along the side of the abdomen. It takes about a day. It ends by a low movement on the long axis which brings again the ventral surface of the mbryo and yolk to lie against the concave surface of the egg. In grasshoppers vhich go through a diapause, such as *Melanoplus differentialis*, the first contrac- ions begin before the onset of the hibernation period, but die away and are esumed when development begins again five months later. If blastokinesis fails o occur hatching is impossible. Unstriated muscle fibres which might cause it ave been described (Slifer[5]).

Although blastokinesis is involved only in a fairly limited group of insect eggs ith much yolk (Imms), it shows, taken together with the yolk contractions at he differentiation centre, how important such phenomena may be in some types f development. And it recalls the suggestions made above (p. 146) that even n amphibian development the part played by contractions of fibrils may be at ome stages not altogether negligible.

As regards later hints of dependent differentiation (inductions) in insect bryos there is some evidence that the formation of the intestinal tract may be e result of a dependent differentiation (A. G. Richards). Reith[1], too, has found at in such a mosaic egg as that of the house-fly the rudiments of the midgut are e only parts capable of development beyond their prospective significance. Here, either the stomodaeal or proctodaeal invaginations are absent, the gut will form holly from one or from the other. It appears, too, that muscles only differentiate operly in the presence of the uninjured ganglion of their segment (Kopeć[2]).

2·82. Post-embryonic inductors in insects

We come now to the facts which are known about development after hatching insects. It might be logical to rule out such a subject from the field of this ook, but for the fact that relatively few insects hatch from their eggs in a fully eveloped state. The larval forms of the holometabolous insects are but the rriers of those imaginal discs from which, during metamorphosis, the structure the adult will be formed, and which, in the form of districts, may already have

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The insects are not the only group which have a "revolutionary" development—it occurs o in some cephalopods (Portmann[1]).

existed in the egg. Considerable advances have been made in our knowle the chemical control of these post-embryonic processes, where, neverthele problems of competence and induction continue to be met with.

Berlese's classification (see Imms) best correlates the manifold phenom embryonic and post-embryonic development seen in insects. In the "prot stage, the embryo is incompletely differentiated, both externally and inte the head and thorax are segmented and have rudimentary appendages, b abdomen lacks them and is poorly segmented or not at all. In the "pol stage, the abdomen has become completely segmented with appendage tracheal system has been laid out, and other internal organs are well d Last is the "oligopod" stage, in which the appendages of head and thorax their definitive form, the tracheal system is completed, and the abd appendages resorbed except such as are to be retained by the adult.

Hemimetabolous insects pass all these stages within the egg, and eme a post-oligopod stage called a "nymph". They then pass through a succ of stages (the "instars") separated by moults, at the last one of which deep-seated changes take place, leading to the fully adult form. Holometa insects hatch at various earlier stages, either in the protopod stage a embryos (e.g. the primary larvae of the endoparasitic Hymenoptera), or polypod state (e.g. the caterpillars of Lepidoptera), or in the oligopod (e.g. the campodeiform larvae of Coleoptera), or finally in a degenerate oli state which is apodous (e.g. the larvae of Diptera). The more precocious th in which the organism emerges from its egg, the more far-reaching the adapt for free larval life have to be, and it naturally follows that the reorganisat the pupal stage (what we commonly think of as insect metamorphosis) is more severe than the passage from the last nymphal instar to the adult fo hemimetabolous insects. The whole process from the pre-protopod state t of the adult is, as Richards & Miller rightly say, continuous and indivisib

The most striking feature of the development of the post-oligopod sta hemimetabolous insects is the succession of moults which they go thr When the physiology of these had been carefully described (Wiggleswo it proved possible to show that a glandular hormone or circulatory ch substance is involved and to trace it to its point of origin by decapitations at varying times during each instar. Wigglesworth[4] found that some organ head produces a hormone, not species-specific among the bugs investi (*Rhodnius*, *Triatoma*, etc.), which induces the moult, by stimulating the petent epidermal cells. The organ is brought into activity by a nervous excit derived from proprioceptive fibres which register abdominal distension c by the food. When the heads of two such blood-sucking bugs have been c the bodies may be joined together at the cut surfaces; if the epithelia heal, mo will be simultaneous, if not, it will nevertheless take place, depending o concentration of hormone present in the partners, i.e. on the time relati feeding, decapitation, and joining with the second insect.*

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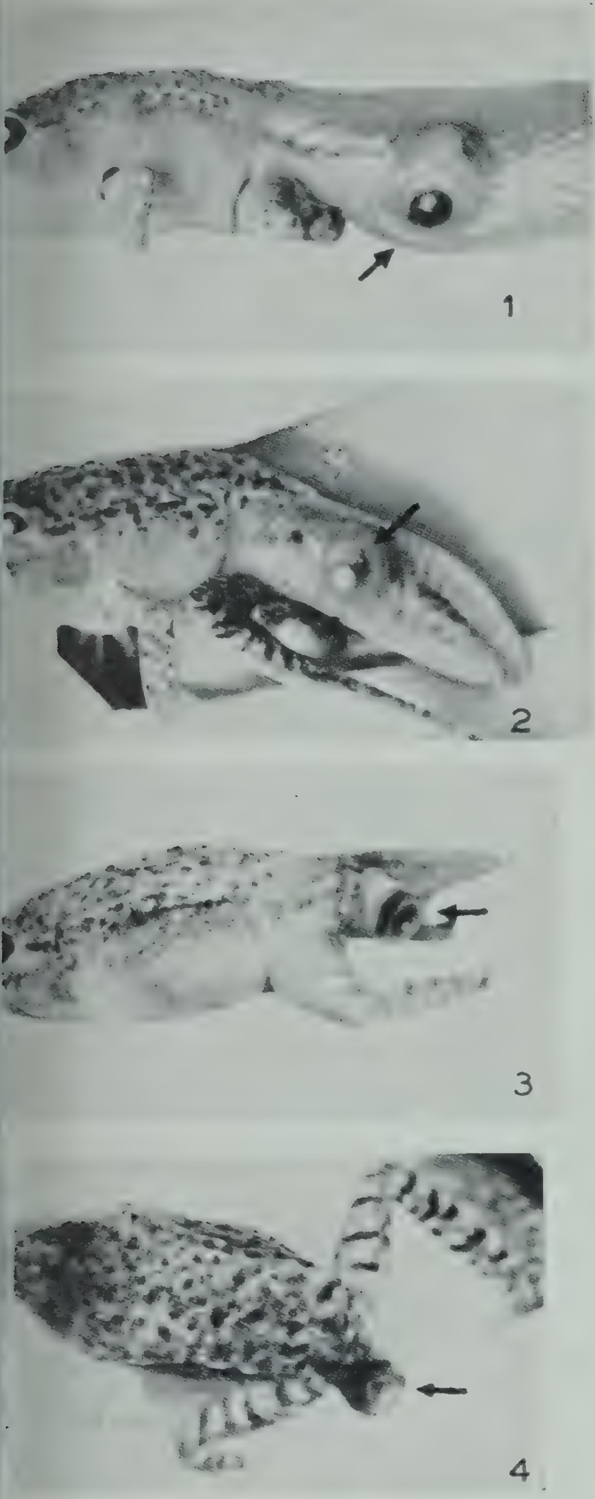
* The two animals may be separated by a glass tube through which the substance diffus Fig. 262.



Resistance of the eye to atrophy when trans-
d to the anuran tail before metamorphosis.

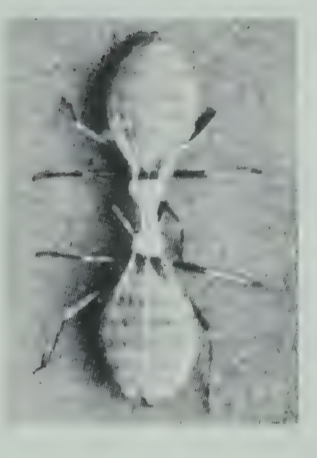


Fig. 262. Two decapitated 4th-instar nymphs of *Rhodnius* united by a glass tube.

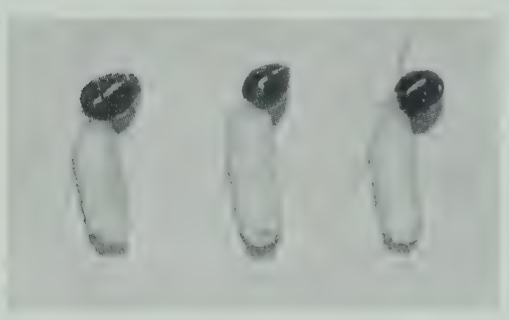


Fig. 263. After ligation of the blowfly larva (*Calliphora erythrocephala*) pupation proceeds only in the anterior portion (see p. 467).

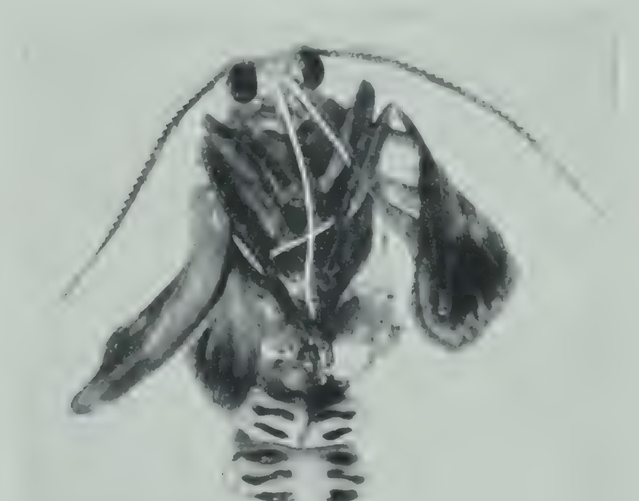


Fig. 264. After ligation of the moth pupa (*Phryganidia californica*), imaginal development (metamorphosis) proceeds only in the anterior half (see p. 468).

The change from the last of the nymphal instars (the fifth) to the adult form netamorphosis) is less straightforward. The simplest hypothesis would be ıat at the last (metamorphic) moult, the same or some other organ would ırow into the circulation a new substance, different from the former. But the cts did not at first lead Wigglesworth[4] to this conclusion. That competence for the ıoulting hormone exists at the last (metamorphic) moult, could easily be shown experiments in which fifth-instar nymphs lacking hormones (because of decapition shortly after feeding) were joined with fourth-instar nymphs containing ɔrmones; the fifth-instar ones moulted instead of metamorphosing. But blood om a fifth-instar nymph transfused back into a fourth-instar or even into a firststar nymph, produced not nymphal moulting but metamorphosis, so that in ıe latter case an extremely minute adult was produced. Competence for metaorphosis must therefore be present from the beginning of nymphal life. Someing seems, however, to inhibit its expression. And that an inhibition is volved rather than the production of a new substance at the fifth nymphal star was suggested by the remarkable fact that if fourth-instar nymphs lacking ɔrmones (because of decapitation shortly after feeding) are joined with fourthstar ones containing hormones, both undergo metamorphosis, though neither ɔuld normally have done so. Wigglesworth[4] explained this and similar experients by saying that at no time does the inhibitory factor reach a sufficient ncentration in the blood to prevent the metamorphosis of a second insect. The nverse to the production of very minute adults, i.e. the production of giant mphs, was also successfully obtained.

From the above work it seemed that the head produces not only the moulting bstance but also a metamorphosis-inhibitory substance. Simple decapitation any nymphal instar produces a certain number of nymphs which undergo ɛtamorphosis, developing into miniature adults; this was explained as being due the secretion of the metamorphosis-inhibitory substance slightly later than moulting substance. In a certain number of cases, therefore, decapitation occur after a sufficient amount of the moulting substance has been formed before there is enough metamorphosis-inhibitory substance.

Vigglesworth's most recent view[6] is that the real nature of the inhibition may that in nymphal moulting the cuticle is quickly laid down, whereas in metarphosis this process is delayed so that there is time for the more elaborate ıodelling of the epidermal and other organs on which the assumption of the lt state depends. He now speaks of two inductors, the nymphal moult mone and the metamorphic hormone, and of a competition between them for stery of the competent tissue, the results of which are experimentally variable.* believes that the metamorphic hormone is produced in the *corpora allata*, nute glands situated above the oesophagus posterior to the oesophageal ıglion, while the moult hormone is produced in the brain, especially the ıtocerebrum which contains Hanström's cells. He draws attention to the

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his is a relief, since in general one should be reluctant to have recourse to "inhibitory factors". y occupy the same methodological *demi-monde* as "reactive forms" of chemical substances in reactions.

similarity in position of the differentiation centre in development at the protopod stage and the *corpus allatum* at the oligopod stage. This may be coincidence, but in holometabolous insects, to which we shall return in a mo the work of Hachlov and others has shown that visible differentiation spreads from a centre in the thorax. Of the chemistry of the two subst nothing is as yet known.

Interesting from the standpoint of this book is the light which the horn control of moulting and metamorphosis throws upon prothetely and metath (phenomena already referred to in another connection; p. 239). Larvae sionally develop typically pupal structures (prothetely). Pupae sometimes r typically larval structures (metathetely). Many such cases have been descr as by Goldschmidt[5]; Pruthi[1] in mealworms; Chapman in *Tribolium*; v. Lenge in other coleoptera; du Bois & Geigy in *Sialis*, etc. Now the production of characters in a first-instar nymph by decapitation, just mentioned, is experim prothetely, and conversely, the production of giant nymphs which may imperfect adult characters is experimental metathetely. By the interpla the nymphal moult hormone and the metamorphosis hormone in varying centrations, all permutations and combinations of morphological effects it is thought, be produced.

Similarly, if the development of the pupa is disturbed by centrifuging operations on the larva, histogenesis may be retarded and the adult may em with its internal tissues still in a stage characteristic of the pupa, accordi Guareschi[4] (working on the beetle, *Lina*).

In the holometabolous insects, larval moulting is also almost certainly contr by a diffusible substance, but less is known about it. As it proceeds in sta animals, moths, for instance, such as *Tineola* (Pruthi[2]; Titschak), the stimul the secretion of the substance must be different from the mechanism in *Rhod* The best proof that a substance is involved is contained in the experimen Bodenstein[1] — analogous to those of workers on amphibian metamorphosi 449) — in which legs from *Vanessa* caterpillars were transplanted on to you and older caterpillars of the same instar, with the result that moulting occurr both host and graft simultaneously. The site of production of the hormon not been ascertained.

A remarkable fact here is that the tissues of the adult conserve their compet to react to the moulting stimulus long after it normally occurs. Furu transplanted the extremities of adult *Anisolabis* to larvae and saw that they mo there; Mauser had the same experience with *Dixippus*. In *Rhodnius* Wigglesw could get the adult to moult by injecting into it blood from nymphal stages Piepho[2] obtained the most striking results by implanting into the fat-bo young *Galleria* larvae bits of epidermis from old larvae ready to pupate. forming balls of skin inside out (cf. human ovarian dermoid cysts, p. they went through all the larval moults over again, the cast skins colle concentrically in the cyst. Pupal epidermis was willing to do the same t What a contrast this is with the loss of competence of adult mammalian ti (cf. p. 174).

Conversely, the tissues (skin) of the young caterpillar shortly after hatching ossess competence to react to the pupation stimulus long before it is normally nanifested (Piepho[2]).

After earlier uncertainties we know now that pupation is definitely under ormonic control. Kopeć[1] extirpated the supra-oesophageal ganglion from cater- illars of the gypsy-moth *Lymantria*, and found that no metamorphosis followed. igaturing experiments then showed that the ganglion, or a site near it, was the ource of the diffusible substance causing pupation.* Similar experiments have een carried out by G. Fraenkel on the blowfly *Calliphora*, Bodenstein on *rosophila*, Geigy & Ochsé on *Sialis*, etc., so that metamorphosis-inducing ormones are now known for the diptera as well as the lepidoptera and hemiptera Fig. 263, opp. p. 464). The flies contains no organ which can be homologised ith the *corpus allatum*,† but Hadorn & Neel[2] and Burtt showed that Weismann's ing is the source.‡

Progress has been made with the chemical identification of the pupation ormone by Becker & Plagge[2], who were able to separate it completely from the -hormone which governs eye-colour (see p. 416). A quantitative test was worked ut using various dilutions injected into tied-off abdomen halves. The hormone non-species-specific, easily dialysable, soluble in water, alcohol and acetone, ut not in chloroform or petrol-ether; stable to heat and acid but not to alkali. elow a critical temperature (6° C. in *Ephestia*) it is not produced at all (Caspari[2]).

It was mentioned above that larval tissues possess competence to react to the upation stimulus long before it normally occurs. Evidence that the pupal blood ossesses hormones which that of the larva does not has been thought to arise om the work of Frew, who claimed that imaginal discs would evaginate *in vitro* the medium contained pupal lymph but not if it contained only larval lymph. is unfortunate that this work, which stands alone in the literature, was not lequately described and illustrated. There is no doubt, however, that larval aries (S. Fukuda[1]) and eye-discs (Bodenstein[10, 13]), when transplanted pre- iturely into pupae, differentiate earlier than they would otherwise have done. it this does not show that any hormone other than the pupation hormone is ncerned.

Some investigators (e.g. Kühn and Piepho) have postulated a second hormone verning the transition from pupa to imago just as the hormone of the *corpus atum* or brain governs the transition from larva to pupa. It is indeed true that aturing the pupa will prevent the posterior half from metamorphosing further, cluding any imaginal discs grafted into it, while the anterior half continues to do (see Fig. 264, opp. p. 464. from Bodenstein's work[8] on the Californian oak-moth).

In a later paper, Kopeć[3] doubted his previous interpretation, but subsequent work has own him to have been right. Caspari & Plagge, using sphingid moths, obtained quite parallel sults, and by implanting a ganglion into a de-brained caterpillar, restored to it its power to ipate. Injection of the pupal blood will also do (Plagge[5]).

It appears that the functions of the *corpora allata* are numerous and important, but still scure (Weed-Pfeiffer; Kühn & Piepho, on *Melanoplus*; Pflugfelder on *Dixippus*; Bounhiol and igge[5] on the silkworm and other moths).

f. nervous tissue as the source of other diffusible chemical stimulating substances — acetyl- line, sympathin, etc. (cf. p. 211).

But when Bodenstein[8] applied the glass-tube method of Wigglesworth t
problem, no diffusible stimulator passed over, and no head-organ could be f
which would act when implanted into the posterior half. Bodenstein[9, 11] then f
that the posterior half will complete its metamorphosis if subjected to conditio
high oxygen-tension, a fact which makes it probable that the occlusion of tra
system and spiracles, rather than any endocrine organ, is responsible fo
effects produced by ligaturing pupae. Under conditions of asphyxiation,
organs suffer more than others, e.g. skin more than ovaries (Bodenstein[6]).
is analogous with the work of Wolsky[2] on localised inhibition of develop
caused by localised CO inhibition (see p. 473).

In this connection we may recall the transplantations made by Byti
Salz[1] of non-developing hybrid moth pupal organs into normal pupae, w
they developed normally and completely (cf. p. 361). Conversely, no
pupal organs transplanted into non-developing pupae died. The lethal
may therefore well have been upon the oxygenation conditions in the h
pupae.

This brings us to the subject of the determination of the imaginal dis
holometabolous insects. As Fig. 265 shows (reproduced from Tower's descript
the imaginal organs are, to begin with, nothing more than ectodermal thicken
on the larval body. From the review of Bodenstein[4] we learn that these ectode
thickenings are still quite undetermined at the beginning of pupation. L
pieces may be removed from the wing discs, for example, without affecting
form of the wings subsequently produced (Meisenheimer; v. Ubisch[1]), but i
extirpation of the organ district is too large, the wing may be suppre
(D. M. Steinberg). This is analogous to the experiments of Harrison[3] (p.
in which discs of varying somite breadths were removed from the limb-
district in amphibia. The determination of colour patterns, wing-vein patte
etc. has already been discussed in the section on the kinetics of gene a
(p. 403); there it was stated that these forms may be affected only du
specific periods in late larval and pupal development by the action of exte
temperature and similar factors. Bodenstein's review[4] gives details of t
specific periods.*

In the moth *Vanessa* the imaginal discs for the legs of the adult are conta
in the proximal segment of the anterior caterpillar legs. Bodenstein[2], amputa
caterpillar legs at a point half-way along the proximal segment, obtained
adult moth legs smaller than usual but of normal form. Removal of the w
segment suppressed altogether the adult moth legs. The regulation poss
moreover, did not extend to the production of an adult hind-leg from a caterp
fore-leg grafted on to a caterpillar hind-leg. In this case *herkunftsge*
development follows.† These results recall the regeneration of amphi

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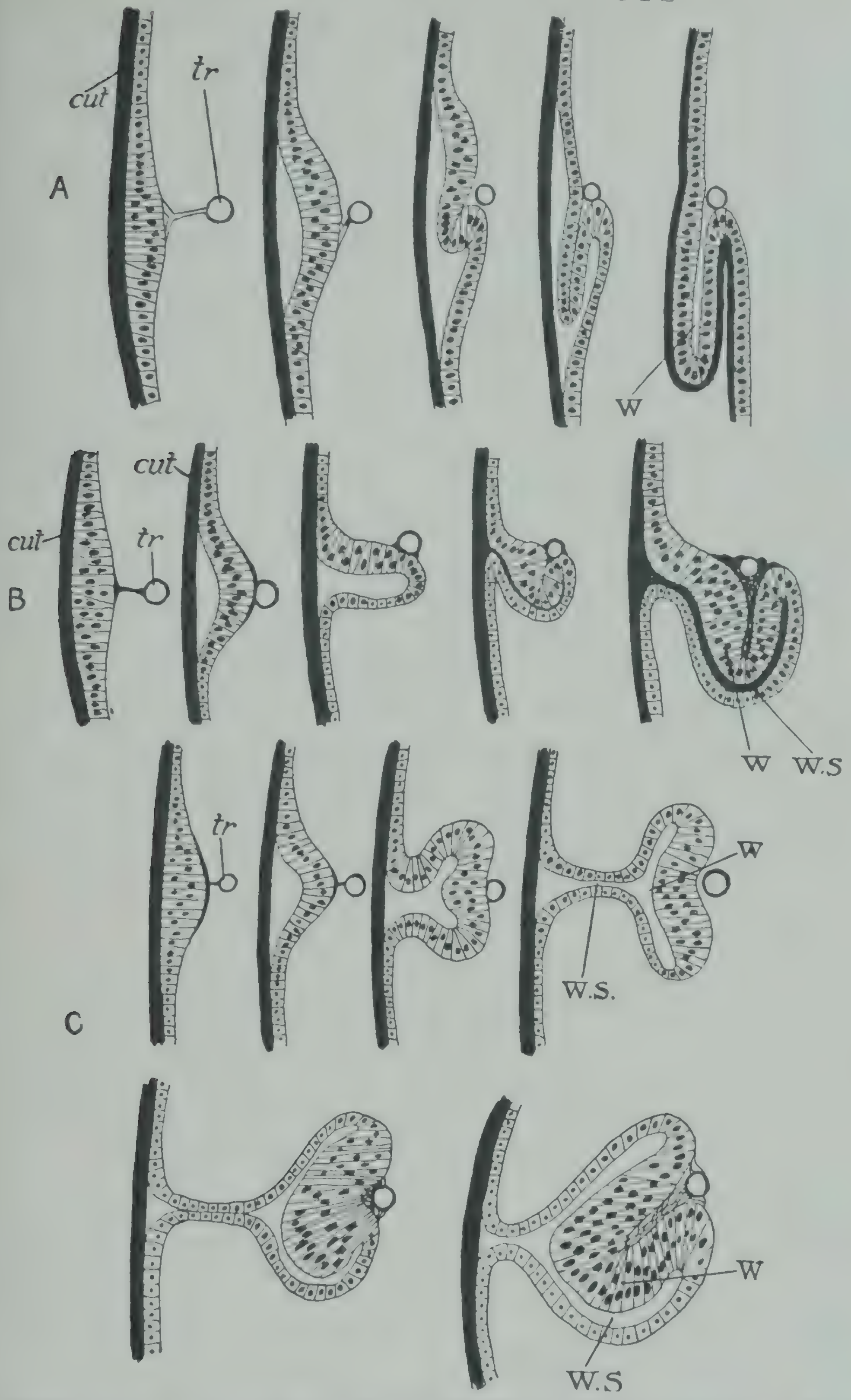
* Nature seems to perform an interesting defect experiment in the parasitism of the pupae
ant *Pheidole* by the larvae of the fly *Orasema*. The parasite removes much material from the an
end of the host, which eventually produces an ant imago with microcephaly.

† Though there is a growth effect, making the transplanted member more resemble a hin
in size.



ɪ. 265. Development of the imaginal bud of the wing in various insects. A. Orthoptera. B. Lepidoptera. C. Diptera. *cut*, Cuticle; *tr*, trachea; *w*, wing rudiment; *ws*, wing sac.

tremities, where great regulation is possible within the organ district itself, t not the indifferent exchange of organ districts.* Determination is not long

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Regeneration of caterpillar legs followed a number of the rules already noted (pp. 430 ff.) he case of vertebrates (Bodenstein[2]). Lateral defects are not made up; the stump has to present and controls the axial determination of the regenerate; the new growth may be supssed by covering with hypodermis, etc. etc.

delayed, however, for Bodenstein[3] could produce double and triple malformat
by inversions of parts of the disc material before any visible differentiation
taken place.

Nothing in the genetical literature or in that of the experimental morpho
of insects gives us any clue regarding the mechanism of determination in
imaginal discs. It may be that further advances in the study of the diffu
substances governing pupation will elucidate the induction of the adult struct
from the competent imaginal discs. Many experiments suggest that the ons
self-differentiation in imaginal discs, at any rate, depends on one or more
stances present in the pupal internal medium. Further transplantation exp
ments will show exactly how undetermined the discs are before this tri
mechanism operates. At present it looks as if their status is rather tha
amphibian limb-buds at metamorphosis or amphibian limb blastemas in regen
tion than that of amphibian tissue in the gastrula with its vast potentialities

Looking back on this section, it is interesting to note that a rather large pa
what we know about determination in insect development arises from the p
bility of interrupting the longitudinal continuum of the elongated insect egg thro
which stimulating substances have to travel, at varying times (e.g. the tyin
of the activation centre at the pre-protopod stage and the tying-off of the sit
production of the pupation hormone at the pupal stage). This method has to s
extent compensated for the difficulties of transplantation experiments in ins

With echinoderm development we reach yet another widely different sys
Before discussing it, however, it will be logical to interpolate a brief accou
our present knowledge of the biochemistry of insect metamorphosis as a b
ground to what has just been discussed.

2·85. The biochemistry of insect metamorphosis

The metamorphosis of insects has long offered an attractive field for biochem
work. From this point of view its interest lies in the fact that the reorgani
pupa is a microcosm the walls of which are closed to the passage of matter ex
in the gaseous state. It is therefore, like the egg of a bird, a cleidoic sys
(see *CE*, pp. 1613, and here, pp. 48 ff.), and we find that it possesses
characteristic metabolic qualities of such systems, that is to say, an emphasi
carbohydrate and fat combustion, a relative suppression of protein breakdo
and the storage of the nitrogenous end-product in the form of uric acid.
while a good deal is known about these questions, the other desideratu
knowledge of the biochemistry of the imaginal discs themselves, has so far
impossible to obtain. New ultra-micro methods will no doubt before long en
an attack to be made on these also. The biochemistry of insect metamorph
was surveyed in 1931 by D. M. Needham[1] (in *CE*, p. 1685), and about the s
time J. Heller[2], in reviewing his own series of publications, gave a useful
of the literature. There is also the interesting, though not easily accessible, re
by Tirelli[6]. Here therefore we need only make a summary of the principal
chemical events in the metamorphosis of an insect and then mention briefly
more reliable contributions which have been made since that time.

The main outlines of what happens during the metamorphosis of a holometabolous insect may be formulated as follows:

(1) There is a pronounced loss of weight.

(2) The respiratory rate drops to a very low level and later rises again before emergence (the typical U-shaped curve).

(3) There is a marked utilisation of glycogen (stored during larval life) as reserve material.[*]

(4) There is a marked utilisation of fat (stored during larval life) as reserve material.[*]

(5) There is little protein breakdown, but accumulation of uric acid[†] goes on which is left behind with the chrysalis on emergence.

(6) The respiratory quotient is low, indicating a combustion in which fat plays at least a very prominent part.

(7) The histolytic processes may be accompanied by an acidosis (cf. amphibian regeneration, p. 442, and amphibian metamorphosis, p. 452).

These conclusions are illustrated by Table 25, which gives the essential details in round numbers) collected from the before-mentioned surveys, in abstraction from differences of sex, social specialisation, etc. The names of the many writers whose work it rests are not given, since full references to the literature will found there. In every case (lepidoptera three, diptera one, and hymenoptera ne) it will be seen that by far the heaviest losses bear on the glycogen and the t. But whereas it is probable that all the fat is combusted, some of the glycogen probably devoted to the formation of the new chitin—a process quantitatively llowed in the silkworm pupa by Kuwana[1]. Recently, further surveys of

Table 25. *Survey of metabolism in insect metamorphosis*

		Pupation		Percentage loss during pupation	
		Beginning	End		
ilkworm moth, *Bombyx mori*	Glycogen	3	0	100	
	Fat	7	2	72	
	Protein	28	26·5	5	
Iawk-moth, *Deilephila euphorbiae*	N-free extr.	264	11	96	
	Fat	141	77	45	
	Protein	378	249	34	too high, cocoon not allowed for
'ent caterpillar moth, *Malacosoma americana*	Glycogen	0·8	0·05	94	
owfly, *Calliphora vomitoria*	Glycogen	0·63	0·17	73	
	Fat	6·96	3·93	44	
	Protein	No change		0	but small amounts of uric acid
e, *Apis mellifica*	Glycogen	9	0·5	94	
	Fat	6	0·5	92	
	Protein	No change		0	but small amounts of uric acid

Note. The above figures are as follows: for the silkworm, gm./100 individuals; for the hawk-th, mgm./individual; for the tent caterpillar moth, mgm. % dry wt.; for the blowfly, gm. 1336 dividuals; for the bee, mgm./individual.

See, on this, the beautiful balance-sheet experiments of Białaszewicz[3] on the silkworm and those Heller[1,7] on the hawk-moth. Fatty substances tend to be laid down before the glycogen.

See Kuwana[3].

metabolism in metamorphosis have been made (A. C. Evans on the sheep blo *Lucilia sericata*; Patton, Hitchcock & Haub on the blowfly *Phormia reg* Melampy & Olsen on the bee; Yonezawa & Yamafuji on the silkworm; Bu on the potato beetle); as will be seen from Table 26, their results entirely con the previous picture.

Table 27. *Metabolism in insect metamorphosis*

		Pupation		Percentage loss during pupation
		Beginning	End	
Silkworm moth, *Bombyx mori*	Total carbohydrate	23	18	47
	Fat	121	68	44
	Protein	33	28	15 to uric a
Sheep blowfly, *Lucilia sericata*	Total carbohydrate	80	14	83
	Fat	240	60	75
	Protein	797	743	7 to uric a
Mealworm beetle, *Tenebrio molitor*	Fat	13	8	38
Potato beetle, *Leptinotarsa decemlineata*	Fat	3·45	1·54	55·5

Note. The above figures are as follows: for the silkworm, gm./1000 individuals; for the s blowfly, mgm./100 individuals; for the mealworm and the potato beetle, % wet wt.

It must not be supposed that the whole of protein metabolism is suppre or unimportant during metamorphosis. On the contrary, the most spectac part of the process, the spinning of the cocoon (as in the silkworm, for insta and later on the final moult, are proceedings in which protein metabolis mainly involved. There is also the fundamental breaking-down of the la structure and the development of the imaginal discs at the expense of the produ But the important thing is that this transfer of protein building-stones is effe with great efficiency, little waste nitrogen (in the form of uric acid) being produ Percentage loss of protein in Tables 25 and 26 refers only to such waste nitro That it should be so small an amount, and that it should appear as uric aci in agreement with the known properties of cleidoic systems.

The loss of weight during insect metamorphosis is almost entirely due to of water by evaporation. Elaborate data for this are available from Kuwana[2] many other authors, and Heller[5] showed that the rate of water-loss is proporti to the external humidity (cf. that other cleidoic system, the hen's egg, wh behaves similarly, *CE*, p. 876).

The U-shaped curve of respiratory rate in metamorphosis has been confir so often that it has become a commonplace (see e.g. Ludwig[1] on the Japa beetle *Popillia japonica*; Janda & Kocián on the mealworm *Tenebrio*; Hitch & Haub on the fly *Phormia regina*; Poulson[1] on *Drosophila melanogaster*; D zhansky & Poulson on the *pseudobscura* species; Balzam[1] and Bell on *Lymantria* *Bombyx*; Zolotarev, Lavrova & Tokareva on *Antheraea*, and Crescitelli on bee moth *Galleria mellonella*).* The respiratory quotient of approxima 0·7 is also always obtained (Ludwig[1]; Hitchcock & Haub; Poulson[1]; Cresci Balzam[1]).

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* Contrary to what is generally found in developing systems, the total oxygen consumptio pupae seems definitely smaller at intermediate than at high or low temperatures (Kozhanch & Maslova).

Though the older results of Bodine & Orr, who found no difference in espiratory rate between wild-type and *vestigial Drosophila* pupae, are of interest, he persistent belief that sex differences in respiratory rate have been established ıust be treated with scepticism. Male pupae consumed more oxygen than emale ones in Poulson's work, but only in one experiment out of two, and the ifference in the same direction found for adult flies by Gowen[1] is the diaietrical opposite of the difference reported by Kucera. There is much Japanese ork also on sex differences, but it is unconvincing and will not be reviewed ere. Melampy & Willis have at any rate established that the queen bee larva nd pupa respires more intensely than that of the worker bee.

Of greater interest is the significance of the U-shaped curve. The most imple-minded explanation of it, namely that it represents the metabolic conequences of complete histolysis followed by new tissue differentiation, as from olk, was criticised by D. M. Needham[1] and cannot be true. Histolysis nd imaginal differentiation are simultaneous processes. As Wigglesworth[3] says, e imaginal histoblasts begin to proliferate rapidly; then each tissue that is to uffer histolysis disintegrates and dissolves. It is uncertain whether the cytolytic gents come from the dying tissues themselves or whether they are secreted into e haemolymph by the leucocytes or other cells. Sometimes phagocytes seem invade apparently healthy tissues, but usually obvious degeneration precedes eir appearance; sometimes the whole process may go on without the interention of phagocytes; sometimes different methods occur in different parts of e same insect. References to the literature on the histology of metamorphosis ill be found in the papers of Poyarkov; Tiegs, and Ch'ang; and the books of igglesworth[3] and Imms. But it may not be wide of the mark to suggest that e disorganisation, at any rate, of the pupal tissues passes through a maximum d so accounts for the U-shaped curve.*

Since 1931 some new facts have accumulated which we must consider in this nnection. That the respiratory quotient is the same at all temperatures in the ysiological range (Crescitelli), though interesting, does not immediately help . The U-shaped curve is compressed at high temperatures, elongated at lower es. More significant is the observation of Drilhon on a lepidopteran pupa *ttacus polyphemus*) that the alkali reserve follows a U-shaped curve precisely erted. At the beginning of metamorphosis 5·9 vol. % CO_2 were found; after hr. in the cocoon there were 28·0 vol. % and after four months the value had nk again to 8·0 vol. %. The most interesting contribution is that of Wolsky[3], who asured the extent of CO-inhibition during metamorphosis in *Drosophila*. Though might have been expected (on analogy with embryonic diapause in insects, q.v., 578) that the respiration in the trough of the U would be unaffected by e inhibitor; the inhibition was light-sensitive and equally marked at all stages, us showing that during the initial decline there is no change in the Warburg-eilin system of respiratory enzymes, nor any change in the saturation of the cytochrome oxidase, but rather a definite destruction of it, followed re-formation. Later in the subsequent rise there was a certain change, but not large and could be explained by secondary effects. By exposing *Dros* pupae to CO and then illuminating them with a narrow beam of light (would reverse the inhibitory effect locally) Wolsky[2] was able to produce a v of regional defects. Depression of respiratory metabolism, for instance, hin the deposition of pigment in the eyes, delayed the development of the wing and reduced the size of bristles, thus producing phenocopies of *stubbl* *stubbloid*. This is the first work in which our knowledge of cell respiration all that that implies, has been brought into contact with the developme imaginal discs and the quantitative expression of genes (see p. 398) further development will be awaited with interest.

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* There are radiographic studies of insect metamorphosis (Heller & Meisels), but owing to the icity of the haemolymph and the pupa-case, nothing very illuminating has yet been derived m them.

The three other directions in which progress has been made are those of on the heat-production of pupae, the composition of the pupal haemolymph the phosphorus metabolism during metamorphosis.

For pupal heat-production we have studies by Białaszewicz[2] and Balzam *Lymantria* and *Bombyx* and by Taylor & Crescitelli on *Galleria*. All the re agree in showing that, as was to be expected, the heat-production declines rises again in the characteristic U-shaped curve. But there are several crepancies. In *Galleria* calculation of the heat-production expected on the of a purely fat oxidation corresponding to the low R.Q. gives a curve keepi a considerably higher level than the experimental one. More informati needed before the meaning of this can be understood. In both insects calorific quotients* are far from the theoretical C.Q. of between 3 and 4 assu normal catabolism of carbohydrate, protein and fat. *Galleria* pupal C.Q. ra from 1 to 2·8, and while that of *Lymantria* and *Bombyx* in the larval stag from 4 to 7, in the pupal stage it is again generally just under 3. No explan is as yet forthcoming for these figures, but Balzam suggests that anae reactions giving out heat may be proceeding during larval development. the authors are forced to the conclusion that endothermal processes exist considerable scale during metamorphosis. It is to be noted, lastly, that d larval life there is at each moult a severe but transient diminution of production (Białaszewicz and Balzam); this gives colour to the idea (whic have already encountered on p. 465 in relation to the course of events i hemimetabolous bugs) that metamorphosis may be, in a certain sense, a exaggerated moult, in which the whole larval organisation, and not merel cuticle, is dispensed with.

The composition of the pupal haemolymph has been studied mostly o silkworm and the hawk-moth (Florkin; Heller[4]; Heller & Mokłowska; D janovsky & Prokofieva; Akao[1]), but also on the wax-moth (Crescitelli & Tay The volume of the haemolymph decreases during metamorphosis from 0· 0·1 c.c./pupa, and there is a percentage decrease of dry matter in it, as als total nitrogen and protein nitrogen. The non-protein nitrogen remains app mately constant (an interesting commentary on the nice balance betw histolytic disintegration and imaginal differentiation).† So does the am ıitrogen and the uric acid (Florkin), but others find that the uric acid of the ıaemolymph falls slightly (Akao[1]). Haemolymph glucose, it is agreed, rises at irst to a maximum and later falls (Florkin; Demjanovsky & Prokofieva), but the ime of the maximum seems uncertain and the values are very divergent. This nay be due to race differences (cf. Heller's results[3] on aberrant races of the hawk-noth below). In the wax-moth, according to Crescitelli & Taylor, there is a naximum of free glucose in haemolymph and tissues at the time of spinning, 'ollowed by a fall and rise to another maximum (not very well established). \ great deal more work will be necessary on many species before we can be said o have a clear picture of the changes in the haemolymph. The establishment or ı peak in glucose concentration would imply that glycogen breakdown at first ›roceeds more rapidly than chitin formation or glucose oxidation. But as Crescitelli & Taylor were unable to detect any glycogen in *Galleria* during larval ›r pupal life, it may be that, in some insects, the carbohydrate is stored as muco-›rotein, or that there is conversion of fat to carbohydrate. Some particulars ıbout glutathione are given by Demjanovsky; during pupation the free –SH alls.

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* $\text{C.Q.} = \frac{\text{heat-production in gm.cals.}}{\text{oxygen-consumption in mgm.}}$ per unit weight per unit time.

† Note also that the changes in peptone and amino-nitrogen observed by A. C. Evans o whole pupa of the blowfly were very small.

Phosphorus metabolism during pupation in *Deilephila euphorbiae* has been tudied by Heller[6], who found the significant fact that there is much less adenyl-›yrophosphate P in the pupa than in the moth, though the arginine phosphate P s not greatly different. It is likely that this is a sign of the muscular disintegration. \lthough Heller analysed a number of organs in the pupa separately for their ›hosphorus partition the subject would repay further study. For example, there eems to be relatively more protein P in the haemolymph than in any of the solid ›arts.

As regards inorganic ions, Bialaszewicz & Landau have shown that the silk-vorm's pupal haemolymph has enormously more Mg″, K′ and Ca″ than human erum and correspondingly less Na′. A large part of the Mg″ is contributed to the ocoon, with a compensatory rise in K′.

The only specific study of fat metabolism is the work of M. Becker on the nealworm *Tenebrio molitor*, incorporated in Table 26. Those interested in the istochemistry of tissues will find a paper on fat in the pupae of this insect by ikolska.

There remains only for discussion the question of acidosis during histolytic generation. The older literature was contradictory, and disagreement still ntinues. Ludwig[3] on the beetle *Popillio* found a change from *p*H 7·07 to 6·79 llowed by a return to 6·94; Booker on the mud-dauber wasp *Sceliphron* found fall from 6·7 to 6·4 followed by a return; Taylor, Birnie, Mitchell & Solinger und the tissues of the bee-moth *Galleria* went as low as 5·53, but late rather an early in pupation. On the other hand the silkworm, studied by Demjanovsky, alzova & Roshdestvenska and by Ongaro[1], showed no acid trend at pupation. is probable that the degree of acidosis depends on the exact nature of the organisation processes, and there is room for a study on much more extensive nes than hitherto comparing the *p*H of the haemolymph at various stages in any different insects with the presence of phagocytosis and other physiological ıenomena.

Pupation may be interrupted by a diapause of varying length (cf. p. 578, where ıbryonic diapause is discussed in detail). In the hawk-moth *Deilephila* *euphorbiae* Heller[3] detected eight genetic races differing in their properties i respect:

	Pupation length days	Glucose in haemolymph %
Two-year	640	—
Latent	300	50
Lethal-latent	Die owing to excessive water-loss	
Protracted	45	—
Subitan	20	50
Rapid	13	—
Lethal soft-wing	Die in pupal stage	220
Lethal black-pupa	Die in pupal stage	380

In pupae showing a diapause the U-shaped respiration curve is correspondi prolonged, diapause supervening at the bottom of the trough. The weight curve is drawn out to an equivalent extent. They are said to differ from *su* pupae in that their metabolic rate depends on the temperature at which pupated, being higher, not lower, the lower the external temperature (Hell As may be seen from Goldschmidt's book ([9], p. 43) length of diapause is inde typically gene-conditioned property.

It will be realised that many interesting questions have perforce been untouched in this short survey. For example, one would like to know whe the metabolism of the pupa is affected by the vast quantities of bacteria c contained, as in the flies, in the larval gut. According to Balzam[2], they are isol in certain parts of the pupa during histolysis, and undergo a rather suc sterilisation at the time of emergence of the imago.

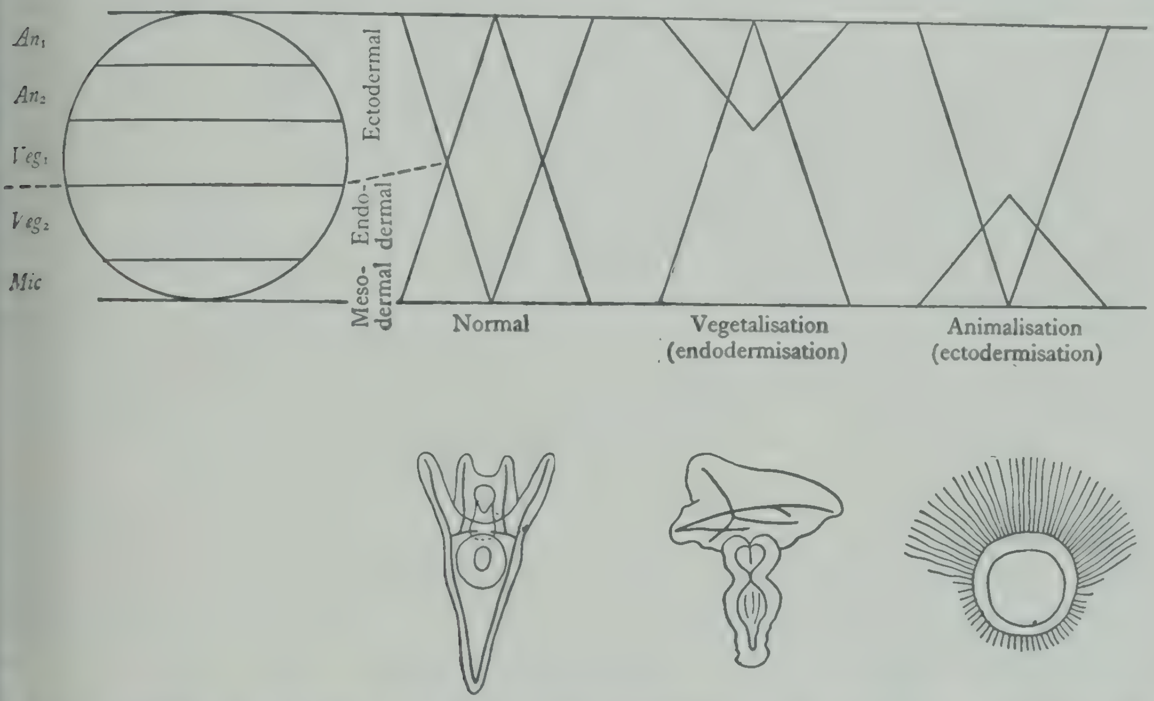
It may be said in conclusion that the typically cleidoic metabolism of the in pupa stands to-day in clearer relief than ever, but that the comparison of chemical with histological and morphological events during metamorphosis offers many unsolved problems. Especially valuable would be an approac the biochemistry of the imaginal discs; their chemical constitution and h auxesis (cf. p. 532), their competence and specificity of response to the pupa hormone, and their capacity for utilising the chemical building-stones set fre the disintegration of the larval tissues.

2·9. Determination in echinoderm development and metamorph

Echinoderms, like insects, develop first into a larval form, which later g rise to the adult by a metamorphosis, though nothing could be more diffe than the plan of organisation on which the two groups of organisms are b Like the insects, too, the echinoderms in their early development have reve to experimental analysis the existence of two centres, vaguely analogous to organisation centre of the amphibia, but not easily to be compared either that or with the two centres of the insect egg. Research on the determina processes in echinoderms has followed more chemical lines than in the cas insects, but owing to the minute size of the eggs of sea-urchins and star-fi the chemical work has largely concerned the action of external substances u the egg, to which it is very susceptible, rather than that of substances whic itself contains. Nevertheless many important facts have been established, mo by the work of the Swedish school, and no praise can be too high for the techn ill and perseverance with which Hörstadius has extended the method of micro-erations to the echinoderm egg, while Runnström, Lindahl, and their col-borators have sought to unite in a unitary hypothesis their numerous biochemical d morphological results.

2·91. The double gradient system

The essential points may be briefly summarised as follows. Two points of diating influence (organiser centres, if you like) are distinguishable in the hinoderm egg, one at the animal pole, the other at the vegetal. As first proposed Runnström[11], their action is best represented in the form of two gradients, one aximal at the animal pole, the other at the vegetal.* Normal development to pluteus depends upon the "co-operation" of these two "gradients", and ny, if not all, of the anomalies of development can be traced to changes in ir intensity. If one centre or gradient apex is depressed then the develop-nt of the embryo veers towards the type "advocated" by the other (see 266). Since the vegetal pole forms endodermal tissues, it will, if circum-nces allow it wholly to control development, bring about a "vegetalisation" "endodermisation" of the embryo. In this case, presumptive ectoderm is de to form endoderm. Conversely, since the animal pole forms ectodermal sues, it will, if given a like control of development by circumstances, bring out an "animalisation" or "ectodermisation" of the embryo. In this case, esumptive endoderm is made to form ectoderm. Extreme but typical forms strating these effects are shown in Fig. 266. When the gradients are equally


An₁
An₂
Veg₁
Veg₂
Mic
Ectodermal
Endo-dermal
Meso-dermal
Normal
Vegetalisation (endodermisation)
Animalisation (ectodermisation)

. 266. Diagram to illustrate the double gradient system in the echinoderm egg (see text).

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he concept of gradients really derives from Boveri's study[2] of polarity in the echinoderm egg 01. The gradient system of echinoderm determination has been graphically formulated with ious refinements by v. Ubisch[5] and Dalcq ([7], p. 150).

powerful, the normal pluteus larva results, with its ectodermal skin, its m chymal spicule skeleton, and its endodermal tripartite gut. When vegetali occurs, much material other than presumptive endoderm is deviated to forr which in this case is seen hanging out from an exo-gastrula, the ectoderma of which is reduced to a thin-walled bag containing abnormally arranged spi Conversely, when animalisation occurs, no gut or skeleton ever forms at al the embryo becomes a thick-walled ectodermal bag provided with a very exc complement of cilia and a hypertrophied apical tuft. These description become clearer in what follows.

Now the characteristic thing about echinoderm development is that changes can be brought about *either* by morphological methods (such as isol and various combinations of blastomere groups, or transplantations) chemical methods (ionic changes in the external medium) *or* by a mixtu both, in which it can be shown that some chemical changes in the medium the same effects as one centre, while others simulate the action of the other echinoderm egg is therefore far more unstable with respect to external influ than any we have hitherto considered. This would not be unexpected in of the nutritional dependence of the embryos of many invertebrates (non-cl eggs) on the external sea-water as regards water and salts (see p. 34), a is significant that it is to ionic changes that the morphogenetic processe most sensitive. The biochemical work has led to conclusions about metab and determination which are not perhaps as yet quite so certain as the fa experimental morphology established by the Scandinavian school.

Some of these facts have been reviewed in embryological monographs, as that of Huxley & de Beer[2];* Caullery[3]; Dalcq[7], etc., but the best review those of Hörstadius himself[6,7], which summarise a large literature, and o biochemical side, that of Lindahl[7]. Here the morphological part will be desc as shortly as possible, but a clear knowledge of it is indispensable for the appr tion of what it has been possible to do with chemical methods.

In the development of the sea-urchin egg (e.g. *Paracentrotus*) the firs furrows are meridional, the second equatorial, so that the egg has eight blastomeres (Fig. 267). The next cleavage is unequal, giving one ring of animal mesomeres, four large vegetative macromeres, and four very small m meres. In the 32-cell stage, the animal half has two rings of eight cells (an_1 and an_2). In the 64-cell stage, the vegetal half macromeres have also di into two rings of eight cells each (veg_1 and veg_2). The egg at this important consists, therefore of an_1, an_2, veg_1, veg_2 and the micromeres, *mic*.†

Each of these groups of cells has a presumptive significance. An_1 will the apical ciliary tuft of the blastula and gastrula and the ectoderm of adjo regions, which is about a third of the ectodermal surface of the pluteus; *an* form another third of the ectodermal surface of the pluteus. Veg_1 (contra earlier opinion) will not invaginate, but will form the remaining third c pluteus ectoderm. Veg_2 alone invaginates, forming all the endoderm, and f

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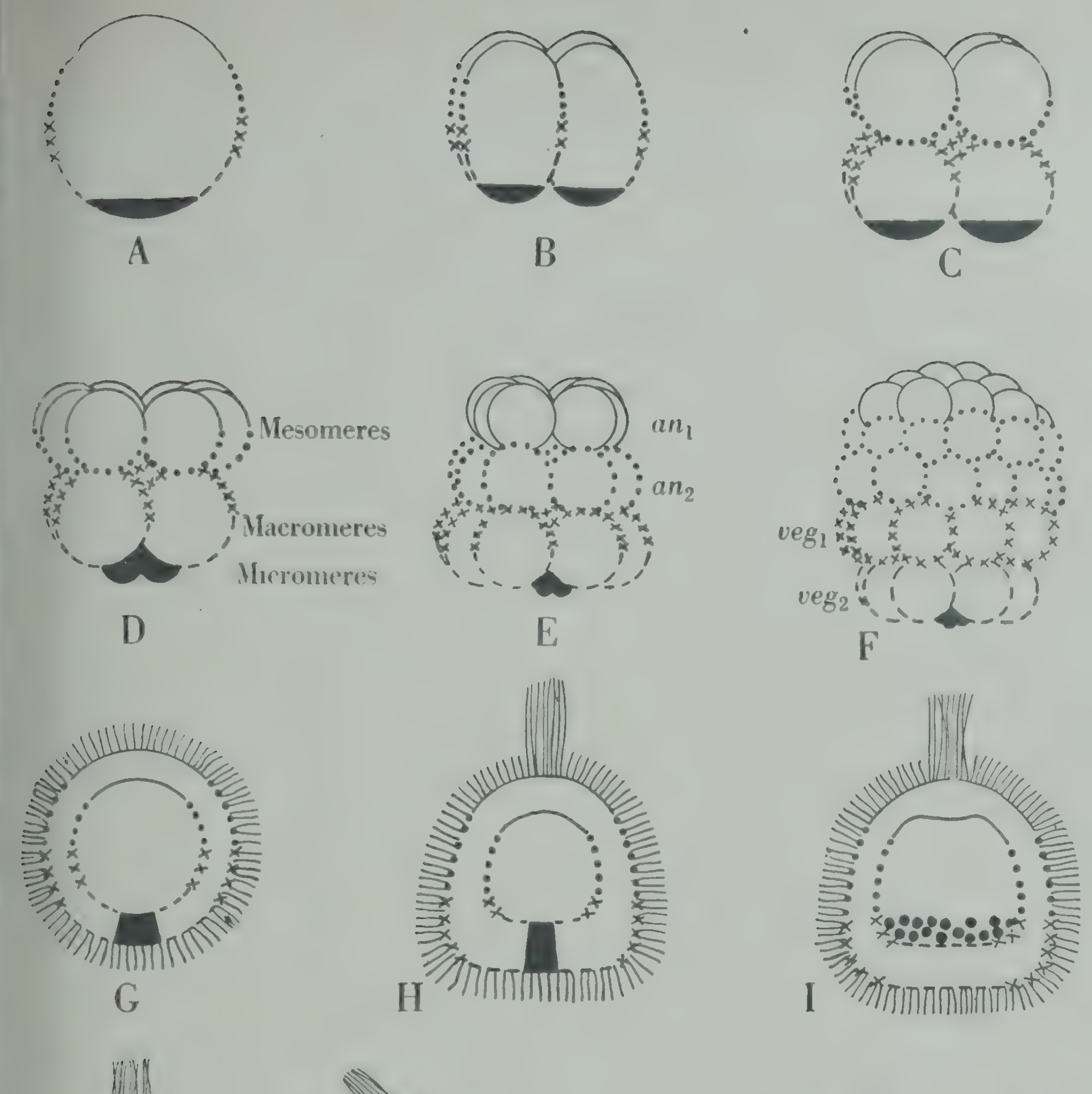
* As Hörstadius has cautioned, parts of an important diagram in the book of Huxley & de (their Fig. 150) are transposed.

† The micromeres derive from the most vegetal part of the egg, even in *Arbacia*, where the been some doubt (Hörstadius[8]).


A
B
C
Mesomeres
Macromeres
Micromeres
D
an_1
an_2
E
veg_1
veg_2
F
G
H
I
K_1
K_2
stom
or
vtr
ar
L
br
oa
aa
ar
or
vtr
br
N
oa
or
aa
M
ar
vtr
br

ig. 267. Normal development of an echinoderm egg (the sea-urchin; *Paracentrotus lividus*). *aa*, Anal arm; *ar*, anal rod of skeleton; *br*, body rod of skeleton; *oa*, oral arm; *or*, oral rod of skeleton; ***stom***, stomodaeum; ***vtr***, ventral transverse rod of skeleton.

A. Uncleaved egg; B, 4-cell stage; C, 8-cell stage; D, 16-cell stage; E, 32-cell stage; F, 64-cell stage; G, young blastula; H, later blastula, with apical tuft; I, still later blastula, after the formation of the mesoderm; K_1, gastrula; K_2, transverse optical section of K_1; L, prismatic gastrula, stomodaeum invaginating; M, pluteus from left side (broken line indicates position of egg axis); N, pluteus from anal side. Continuous lines, an_1; dotted lines, an_2; crosses, veg_1; broken lines, veg_2; black, *micromeres*.

the cells originating from *mic* migrate into the blastocoele cavity before gastr tion has begun, and later give rise to the spicule skeleton. The arms of the plu are induced by the skeletal cells, a "formative Reiz" already detected long by Herbst[1].*

2·92. Experimentally induced deviations from presumptive fate

When Hörstadius[1] studied the development of isolated animal† and ve halves he found that their behaviour was exactly the contrary of what would been expected had the early views of Driesch (see p. 121) been justified. Regul would have given small but harmoniously formed larvae. But instead the influe of the two centres seemed to be freed in such conditions. The animal mat remained purely ectodermal, producing first ectodermal balls or blastulae hypertrophied ciliation and later swimming blastulae with bands of cilia, so times showing a small stomodaeum as the façade to an absent gut. Animal h never gastrulate.‡ The vegetal material showed various types, mostly gut irregularly shaped skeleton enclosed in an oval, mouthless and armless, though the gradient system may re-form so that a proper pluteus is produ Hörstadius[2] noticed, on cultivating the operated embryos together, that the poorly developed animal halves corresponded to the best developed vegetal h and *vice versa*. This he explained by supposing that the animal halves with sto daeum and ciliated band came from eggs in which the third furrow had st through nearer than usual towards the vegetal pole. The material which thus obtained from the vegetal half had inhibited the overgrowth of the a tuft and had *induced* the stomodaeum and the ciliated band. Correspondi the poorly developed vegetal halves of the same embryos would have had animal material than normally. He was thus led to try the permutations combinations of the different groups of cells.

The results are shown in Fig. 268. The animal half alone (an_1+an_2) g nothing but a ciliated ectodermal ball (A3). But $an_1+an_2+veg_1$ gives a cili blastula with a stomodaeum, thus proving the correctness of the view expre above, that the material of veg_1 is necessary for suppression of excessive a tufts and formation of the stomodaeum (B3). More remarkable is the re obtained by the combination $an_1+an_2+veg_2$, namely, a perfect pluteus larva (I Here veg_2 behaves not only in accordance with its prospective significance invaginating and forming the gut, but it also provides the skeleton by induc or regulation. Then if the four micromeres are implanted in an entire presump ectoderm so that we have the combination $an_1+an_2+veg_1+mic$, a perfect plu is obtained (E3). Here the micromeres have behaved not only according to t prospective significance, but have also provided the gut, either by inductio ulation. Induction is more probable, since the volume of material in the romeres is so small. Just the same thing happens, but more strikingly, if the

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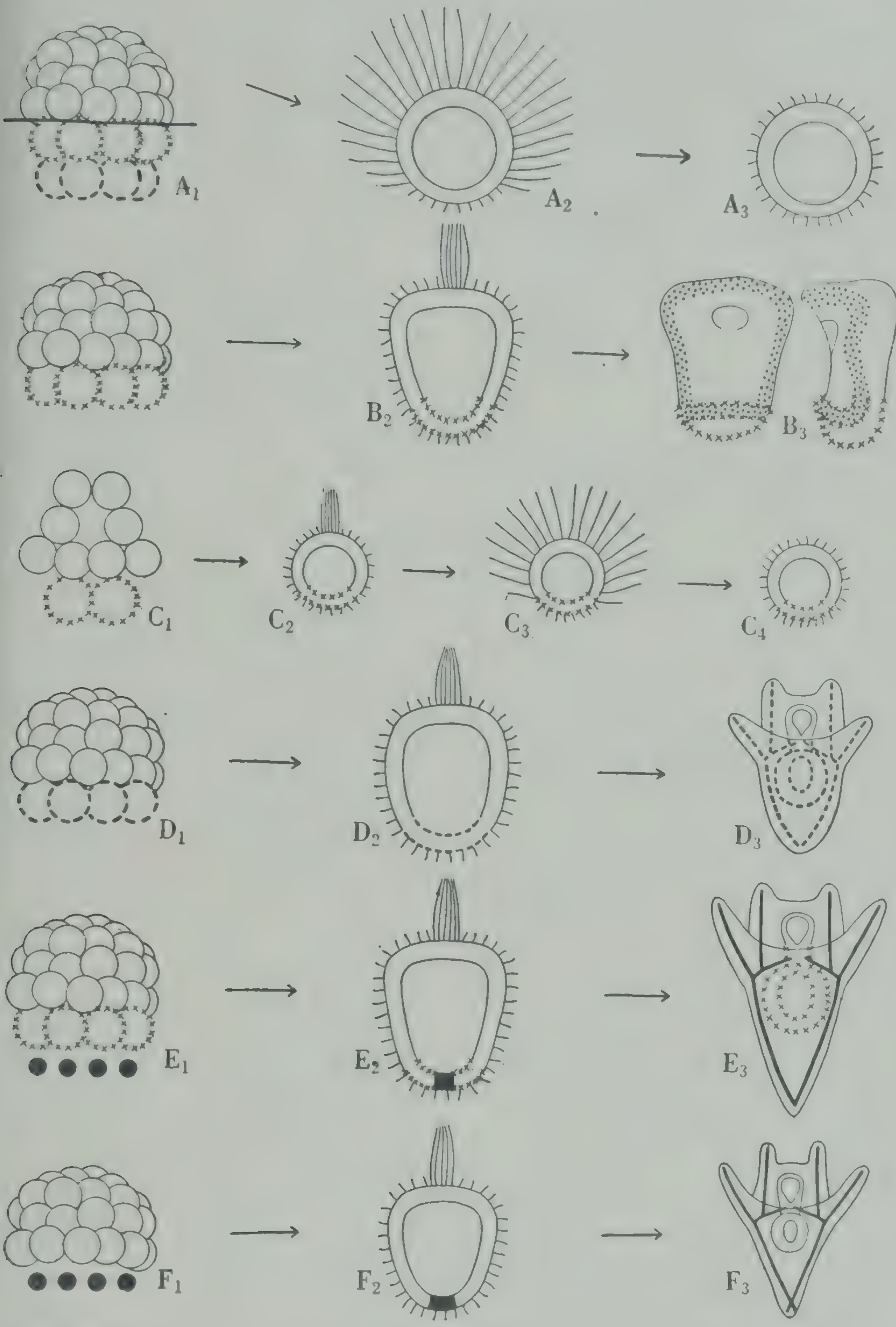
* But the *position* of the arm evaginations is determined earlier, though they will not beg grow out without the skeleton. For the rather complex developmental mechanics of the skel the reader is referred to the papers of Runnström[8]. Its formation can be selectively suppresse Ag′ (Bohn & Drzwina[1]) and intensified by Sn″ (Drzwina & Bohn).

† First obtained by Zoja in 1895.

‡ Hence the echinoderm egg is not a harmonious equipotential system in the extreme sens Driesch. Hörstadius[1] has well said that every part can give rise to every part, but n own power.



ig. 268. Effects of permutations and combinations of the various groups of cells in the echinoderm embryo (see text).

omeres are associated only with an_1 and an_2 ($an_1 + an_2 + mic$), for again a perfect us is formed (F_3). The four micromeres thus have the power to convert even nate[illegible]l to endoderm. But if they are removed, leaving everything else, the e-forms with veg_2 as the most vegetal part, and a normal pluteus results.

In this series of results there is one strange, and as yet unexplained, ph menon. If an ectodermal half ($an_1+an_2+veg_1$) is divided into four *meridi* parts (like the component sections of an orange), so that only two veg_1 cells associated with eight *an* cells, the result (C4) will not be the same as form

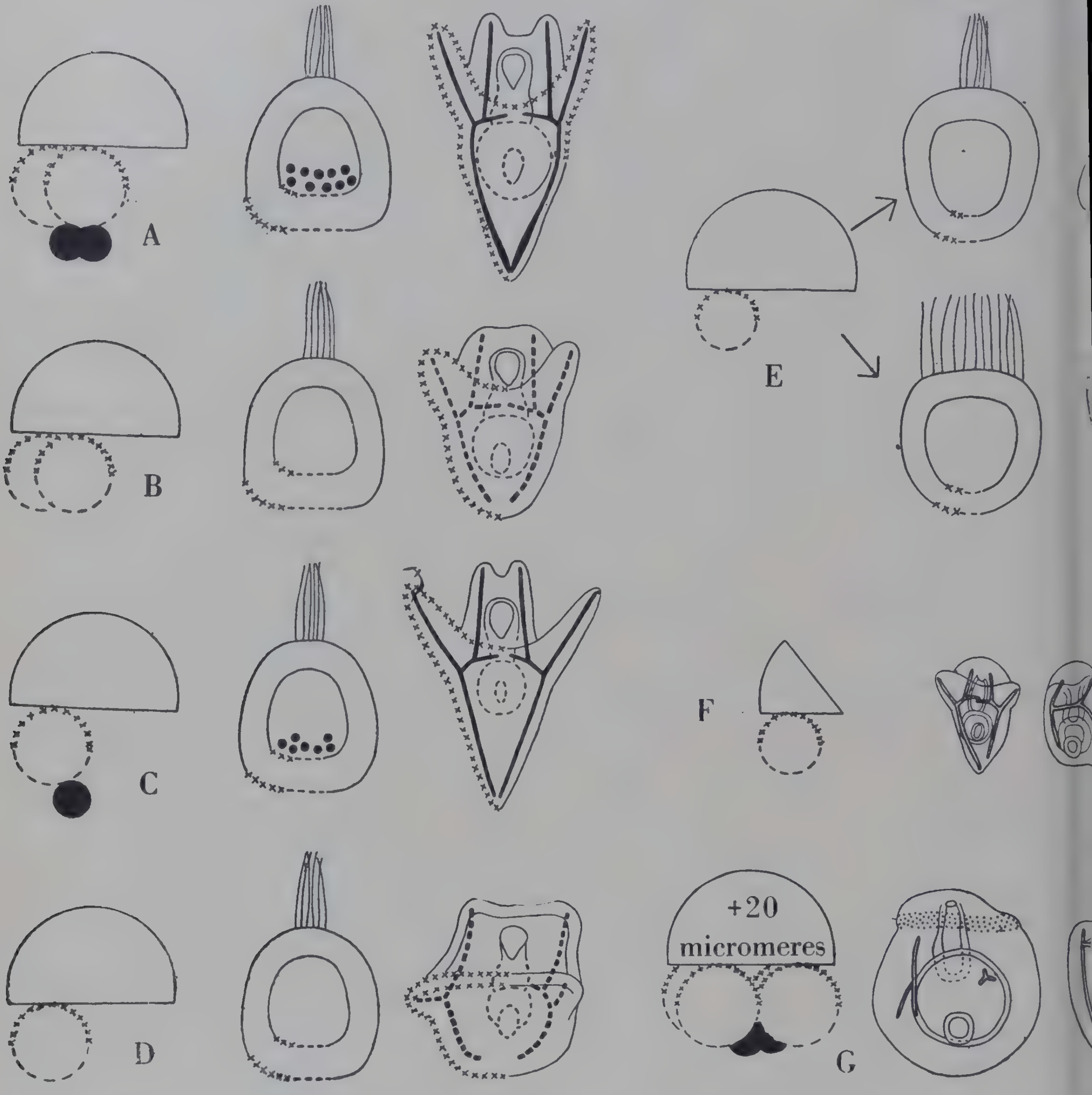


Fig. 269. Effects of progressively diminishing the amount of vegetal material present in tl echinoderm embryo (see text).

(B3). In this former experiment a certain degree of vegetalisation was brou about by the presence of the veg_1 cells (see above), i.e. the suppression of ap tuft hypertrophy and the formation of the stomodaeum; here, however, animalisation forces have it all their own way. Hörstadius[3] concluded that vegetal forces, when quantitatively below a certain level, can no longer resist animalising ones.

This experiment leads on to one of the most important principles established Hörstadius[6], namely that the development of a typical pluteus is dependent, t on the absolute amount of animal or vegetal material present, but on their ative amount. This can be demonstrated (*a*) by gradually diminishing the ount of vegetal material present, and (*b*) by implanting varying numbers of cromeres into different combinations of blastomeres. We will glance first at e former method, then at the latter.

Fig. 269 shows the results of the following combinations: animal halves in ntact with two macromeres and two micromeres, or with two macromeres only, with one macromere and one micromere, or with one macromere only, or ally with only the half of one macromere. There is a definite trend towards imalisation as the amount of vegetal material decreases, so that at the final ge, *E*, animalisation is complete, and the vegetal macromere itself may be imalised. It may also be seen that volume for volume the micromeres are re strongly vegetalising than the macromeres. But now supposing that instead combining the half-macromere with a full quantity of animal cells, the esumptive ectoderm is reduced by two-thirds, the original balance is regained, d a very minute but well-formed pluteus results (F). Similarly, if the amount micromere material is greatly increased by adding twenty micromeres to a rmal embryo, the whole will differentiate as if it were a vegetal half (G).

Fig. 270 shows the results of transplanting varying numbers of micromeres into lated parts of the embryo. The differentiation of the isolated layers, if not erfered with, is shown in the left-hand column. An_1 and an_2 form completely imalised blastulae, and veg_1 almost so. Veg_2, the presumptive endoderm, gives al larvae with a large gut and a few small spicules. This means that it has made ne material more animal than itself (ectoderm) by regulation, and also that it s made some material more vegetal than itself (spicules). In certain isolated gions, therefore, there can be a reorganisation of the gradient system leading new poles.

licromeres when isolated form at first a small blastula, but this eventually ntegrates as they are too vegetative to be able to form any ectoderm by ulation. Now if one, two, or four micromeres are added to the isolated veg_2 embryo will be too vegetative to gastrulate. An exo-gastrula will be formed ilar to those produced chemically (see below, p. 486). But four micromeres ether with veg_1 gives an embryo of the same type as what is produced from isolated. In order to get the most pluteus-like larva four micromeres have e added to an_1; two micromeres have to be added to an_2; one micromere to ; and none to veg_2. This clearly illustrates the balance required between the dient forces. If the animal material is present in great superiority, the vegetal ce of a micromere may be overwhelmed. This happens when one micromere combined with isolated an_1. First the apical tuft hypertrophy is suppressed, t later animalisation gains the day, and no gut or skeleton ever appears.

Another type of experiment consists in implanting the four micromeres at erent levels along the gradient system. Thus in the example shown in Fig. 271 micromeres were implanted into an embryo between an_1 and an_2. A new vegetal centre was formed, which attracted mesenchyme cells and gave gastrular invaginations instead of one, leading in the end to an induced gut

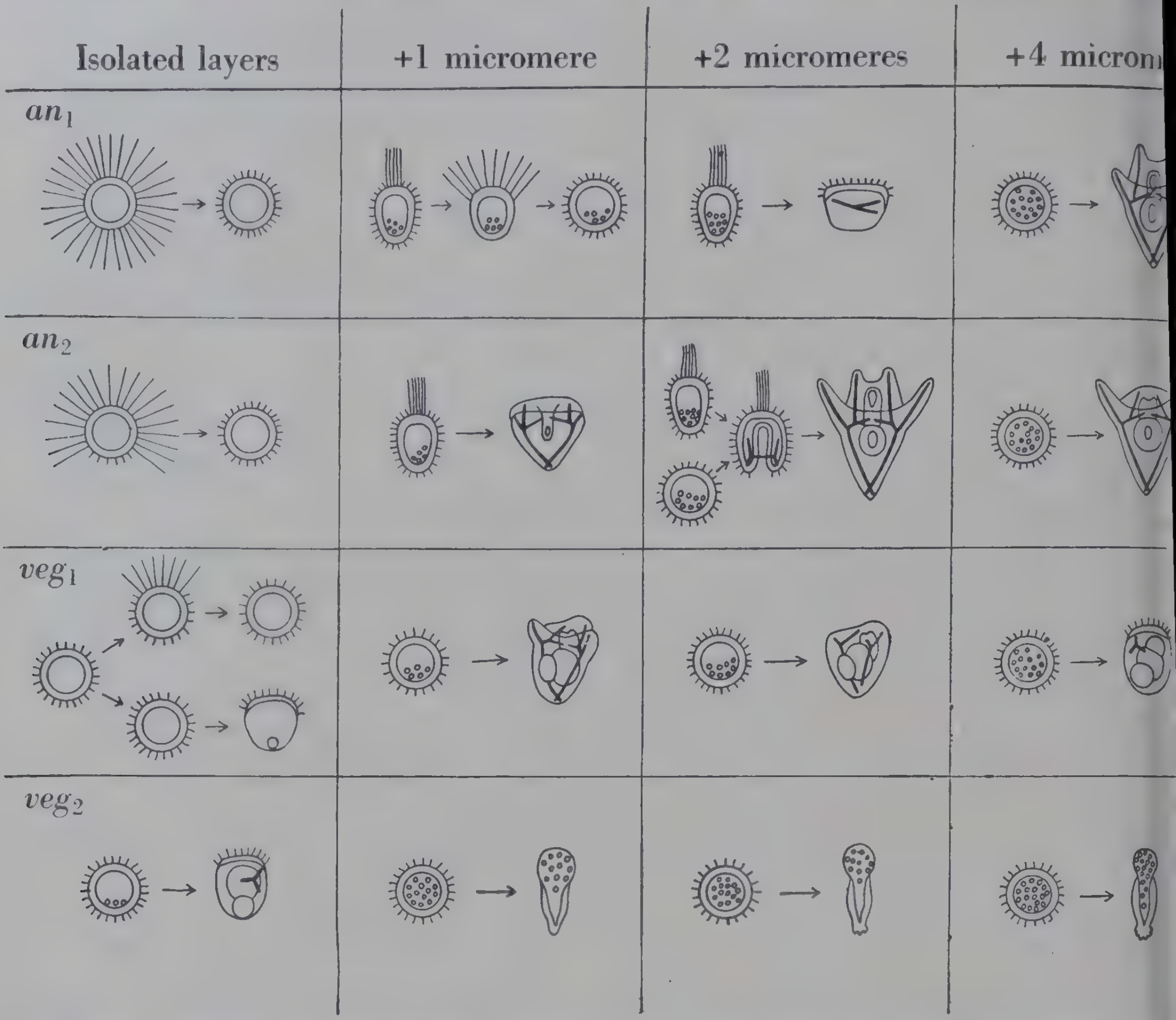

Isolated layers
+1 micromere
+2 micromeres
+4 micron
an_1
an_2
veg_1
veg_2

Fig. 270. Effects of implanting varying numbers of micromeres into various isolated group cells in the echinoderm embryo (see text).

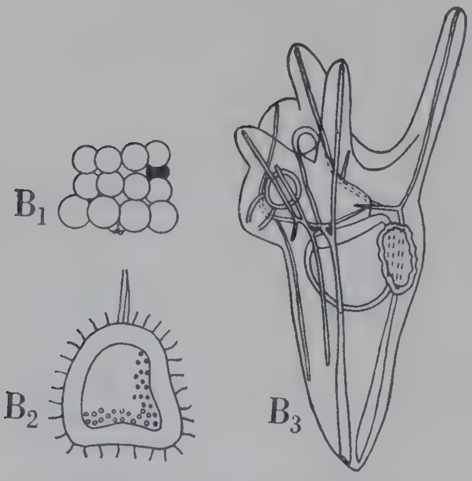


Fig. 271. Implantation of micromeres between an_1 and an_2.

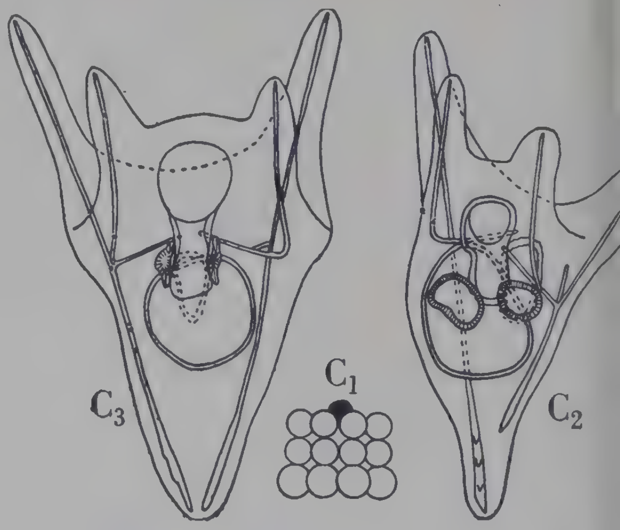


Fig. 272. Implantation of micromeres at the animal pole.

a fairly complete supernumerary skeleton. The nearer to the animal pole micromeres are implanted, the smaller the induction will be, while in the o rection, if they are implanted towards the vegetal pole the induced gut will entually fuse with the host gut.

One of the extremest forms of this experiment consists in removing the micro- eres from the vegetal pole and implanting them at the animal pole (Fig. 272). ere nothing is added to the egg. The result is an enlarged gut and coelom. e micromeres at the animal pole were unable to induce a secondary gut or eleton, but they weakened the animalising tendency so that some material ich normally would have formed ectoderm was deviated to form endodermal rivatives.

The animal-vegetal axis is, as we know, already laid down in the ovary. The imal pole (where the micropyle and the polar bodies are) is opposite to the int of attachment (see Lindahl[2], with literature). This axis must, in a sense, rrespond to the yolk gradient of the amphibian egg, but analogy is difficult and we shall return to it later . 500). The animal-vegetal axis is more stable than dorso-ventral and the dextro-sinistral axes; it is not ected by moderate stretching or by centrifuging (cf. . 497, 668), though a strong constriction may divide it o two. A new axis may be formed in an embryo by the plantation of a new vegetal centre (micromeres, in the periment just described), but the animal centre may also verse or alter polarities in the embryo. If the vegetal lf of an egg be cut off and turned upside down beneath animal half, the latter may incorporate part of the dodermal material within itself by forcing it to gastrulate a direction directly opposite to that of its normal larity. Chemical animalisation, too, may lead to the appearance of a secondary imal centre at what should have been the vegetal pole of the egg, and hence the duction of two apical tufts instead of one (Lindahl[7]). Treatment with hypo- ic sea-water may, furthermore, as Runnström[3] and Newman[2] found, cause the earance of several small supplementary invaginations anywhere on the gastrula . 273). Competence for invagination is evidently widespread. Hörstadius[6] aks of this as an "anarchy of the axis" (cf. multiple gastrulation in amphibia, 57).

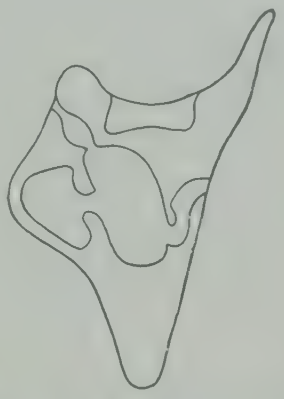


Fig. 273. Supernumerary invaginations in the echinoderm embryo.

93. Chemically induced deviations from presumptive fate

Ve are now in a position to appreciate the effects of various chemical agents echinoderm determination. The initial step was taken in 1892 by Herbst[1] en he found that exposure of developing sea-urchin embryos to lithium salts ulted in uniform exo-gastrulation. Later he studied the effects of excess and ect in all the ions of sea-water in a series of researches (described in *CE*, 1271). Fig. 266 shows one of Herbst's drawings of exo-gastrulae, showing the iation of most of the material of the egg in an endodermal direction (vegetalisa-). If lithium is applied in late stages, after determination is completed, it , of course, no effect.

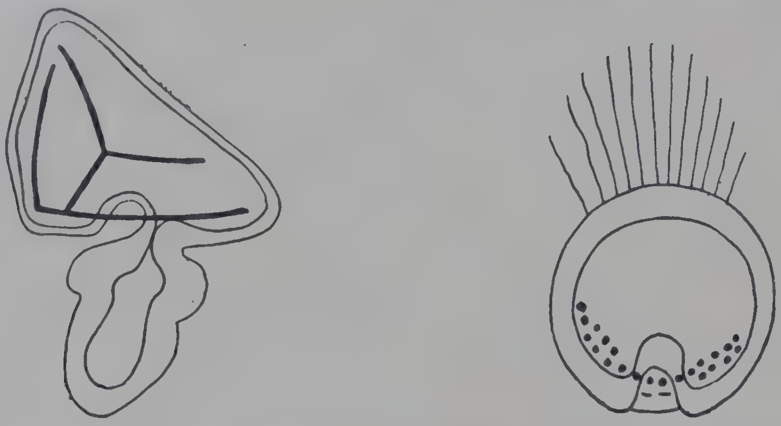
From the work of Lindahl[7], Runnström[14] and others, a table can be draw showing the chemical agents which have been found to have marked vegetal and animalising properties.

Table 27. *Morphological effects of external agents on echinoderm embryo*

Vegetalising agents	Animalising agents
Li'	SCN' (best before fertilisation)
$\mathrm{Li}'+\mathrm{KCN}$ (intensified)	
$\mathrm{Li}'+\mathrm{CO}$ (intensified)	$\mathrm{SO_4}''$-lack
Mg'' (?)	Ca''-lack (slight)*
	I' (best before fertilisation)
	Pyocyanin $+\mathrm{SCN}'$

* Due perhaps to impurities in the reagents (Runnström & Thörnblom).

Among these agents antagonism and reversibility are easily demonstrable. vegetalising action of lithium (seen, for example, in Fig. 274) is reversed by S or by sea-water lacking the sulphate ion, and *vice versa*. Pyocyanin also reve the vegetalising action of lithium (Runnström[15]), while cyanide (Lindahl[7]) carbon monoxide (Runnström[6,11]) intensify it. Here there is a direct connec with respiratory mechanisms. A typical animalising action, not extreme, brou about by thiocyanate, is seen in Fig. 274; the invagination of the endoderm been arrested and the apical ciliary tuft exaggerated.



Intermediate vegetalisation　　Intermediate animalisation

Fig. 274. Vegetalisation and animalisation. The examples in Fig. 266 are more extreme c

In attempting to analyse the metabolic meaning of these effects Lindahl[5] n manometric measurements of the respiratory rate of embryos in the prese and absence of lithium salts.* He was able to establish that whereas du normal cleavage and gastrulation the respiratory rate increased steadily,† in presence of lithium this increase of respiratory was inhibited, so that respira proceeded at a uniform rate, no faster after 8 hr. than during the first hour a fertilisation (see Fig. 275). The inhibition was thus not large (maximally 2

* What is known of the respiratory metabolism during normal echinoderm development w found in Section 3·31.

† This rising curve has been obtained by many reliable observers; cf. *CE*, Fig. 111. Lir wishes to regard it as analogous to the rising curves of fermentation after the "induction" pe but I prefer the older explanation that it is due to the formation of new respiring protoplasm inert yolk. The fact that the respiration is not inhibited at all immediately after fertilisation m according to him, that the animalising substance is not yet being formed.

0·135 N LiCl), but it may be accepted that there is a lithium-sensitive fraction respiration in the sea-urchin embryo.* Up to a late time it is reversible on placing the eggs in lithium-free sea-water. Like the morphological action of ′, it is decreased by the simultaneous presence of excess K′ ions. In brei periments (cytolysed eggs), the reduction time of methylene blue in the presence hexose-monophosphate as donator was considerably retarded by lithium. On ese grounds, Lindahl[7] put forward the hypothesis, which must still be regarded tentative, that the metabolism characteristic of the animal pole of the egg, and nce reduced or abolished by vegetalising agents, was of carbohydrate nature.

Animalising agents also inhibit the respiratory rate, as Horowitz[4] has shown NaSCN. In the same way, Lindahl[7] found that lack of SO_4'' ions inhibits the spiratory rate at the blastula stage, t the fraction so inhibited is not the me as that sensitive to Li′, because inhibitions sum. Suggesting that only function which SO_4'' ions ld be likely to serve was that of toxicating and removing the aromatic ste-products of protein breakdown, framed the hypothesis that the tabolism characteristic of the vegetal le of the egg (and hence reduced or olished or rendered toxic by animal-ng agencies) was of protein nature. is well known, phenol and indoxyl, sing from tyrosine and tryptophane the intermediary protein catabolism the higher animals (not only, as was thought, from bacterial action in gut; see *CE*, p. 1236), are excreted ombination with sulphur; phenyl-hate and indoxyl-sulphate (indi-), forming the greater part of the alled "ethereal sulphates" of the ne. Phenol-sulphatases, of very various origin, plant and animal, especially lusc, are known (see the review of Fromageot). Lindahl[7] then succeeded in wing that sea-urchin eggs do indeed contain a phenol-sulphatase, capable of rolysing 115 × 10^{-4} mMol. phenyl-sulphate/c.c. egg brei (containing 5 mgm. N) 15 hr., and hence presumably of synthesising similar compounds in the sence of sufficient inorganic sulphate. It also acts on indoxyl-sulphate ndahl & Öhman).

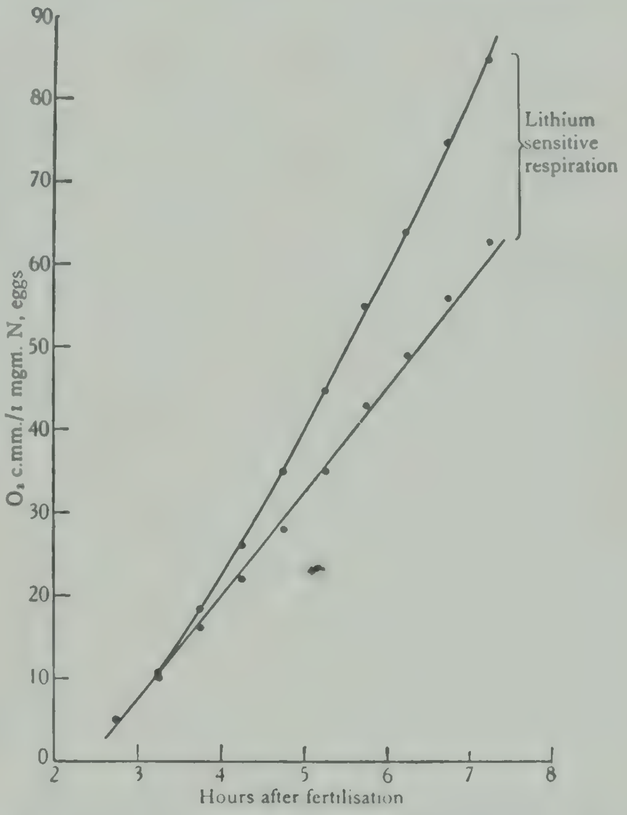


Fig. 275 Respiratory rate of echinoderm embryos in the presence and absence of lithium.

Animalising determination would therefore be associated with carbohydrate akdown, and in the event of this being in some way inhibited, the vegetalising

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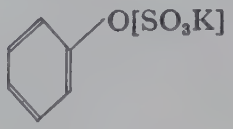
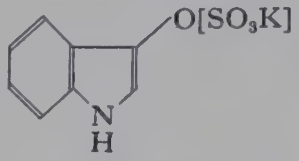
he inhibition is naturally very much larger if calculated only on the respiration-rise above the tant rate.

forces would predominate. Conversely, vegetalising determination woul associated with protein breakdown, and in the event of an inhibition of thi an intoxication by certain of its products, the animalising forces would dominate. Such an interesting hypothesis deserves a detailed criticism,



Phenyl sulphate



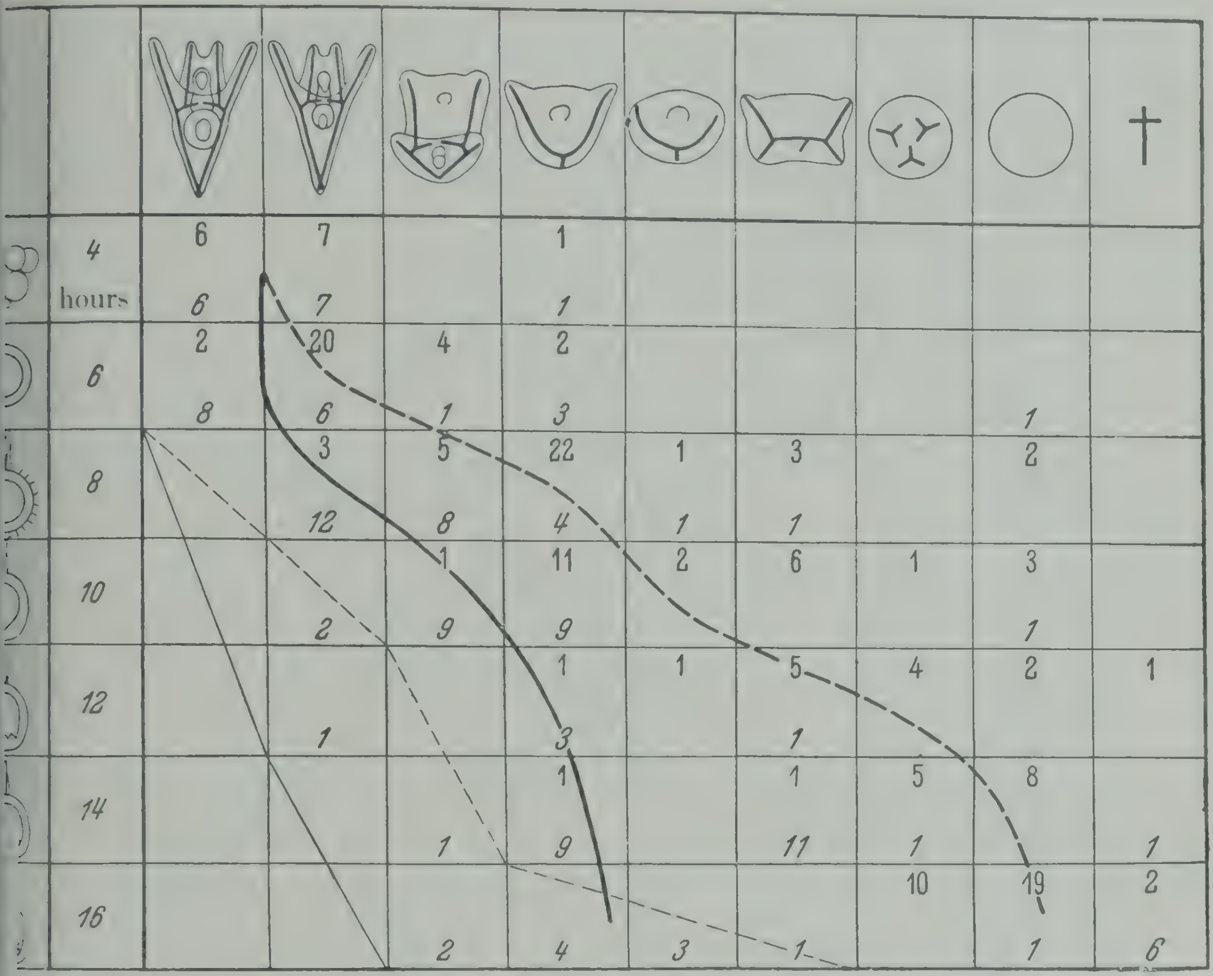
Indoxyl sulphate

before proceeding to this, we must consider the third of the three main cla of experiment referred to at the beginning, namely the use of a mixtur morphological and chemical methods in order to demonstrate their interchan ability.

2·94. Combinations of the experimental and chemical methods

Only two of the most striking mixed examples will be taken. In the fi Hörstadius[4] studied the course of animal-vegetal determination, using microme and lithium in parallel as vegetalising agencies. In the second[5], he was abl show that animal blastomeres, if first vegetalised with lithium, will act as pow fully as the micromeres themselves in vegetalisation.

In the normal whole egg, isolation experiments at varying times after fertili tion show that for the animal half (presumptive ectoderm) determination occ quite early (8–10 hr. after fertilisation). If an_1 is isolated alone, however, det mination is not complete till 14–16 hr. after fertilisation. This shows that influence spreading from the vegetal pole must be at work. The question n arises, how late in development can animal halves react to a vegetalising stimul i.e. the implantation of micromeres or treatment with lithium. Hörstad answered it by isolating large numbers of animal halves in the 16-cell st (4 hr. after fertilisation) and then implanting micromeres into them after lapse of various times (see Fig. 276). In this way many different kinds of lar are obtained (plutei; plutei with small guts; larvae with guts that do not re the stomodaeum; blastulae with a ciliated band, stomodaeum, skeleton and arr similar blastulae without arms; blastulae with skeleton and band only; and fin blastulae with a few spicules or none at all). This is a graded series of vegetalisati Now if the time elapsing between the isolation of the animal half and the implan tion of micromeres into it is long, the effects produced will be poor. If it been relatively short, they will be better. This shows the onset of animalis determination. The shorter the time, the better the effects will be; hence Fig. 276 it is possible to draw a line across the table showing the average effe produced in this way (the thick dotted line). For example, implantation micromeres 6 hr. after fertilisation will vegetalise the animal half to the ext of giving a pluteus, but at 12 hr. a few spicules only will be produced in resulting blastula. An analogous line can be drawn showing the best effe produced in this way (the thick continuous line). If, however, the animal hal e isolated, not at the 16-cell stage but at various subsequent times, and the icromeres implanted into them immediately after their isolation, the results nd to be much better (the thin dotted line represents the average and the thin ntinuous line the maximal effect). Under such conditions, an animal half can ll respond to a vegetalising influence as late as the stage immediately prior to strulation. Hörstadius[4] concluded that the animal half becomes determined ore quickly in the animal direction when it is isolated than when it is all the ne connected with vegetal material.



276. Plot of the course of animal pole determination in the echinoderm embryo, micromeres being used as the vegetalising influence (see text).

Γhe whole set of experiments was then repeated using Li′ as the vegetalising ncy instead of the micromeres. As Fig. 277 shows, the results were very similar. he animal halves were placed in lithium sea-water immediately after isolation, y could be vegetalised until just before gastrulation; whereas if time was owed to elapse after the isolation at the 16-cell stage before the lithium treatnt was begun, the halves were much more difficult to vegetalise.

Such a far-reaching parallelism between the action of Li′ and that of the microres invited a test of the vegetalising power of animal fragments themselves getalised with Li′. Such an experiment is shown in Fig. 278. Hörstadius lated animal halves and vitally stained the vegetative surface of *an*$_2$ (D).

Controls developed into typical ectodermal blastulae with ciliary hypertro (A, B), showing that he had pure animal material in hand. The cells contai the stained material were then put into lithium sea-water (E) and after 24 hr. stained part was cut off (F), stained, if necessary, more deeply (H), and attac to the vegetal pole of another animal half in the 32-cell stage (I), which, if to itself, would have produced nothing but an ectodermal ball and cilia (C, On the next day it was clear that the ciliary tuft was under strict vegetal con and not hypertrophying (M), and before long invagination took place (P), lead

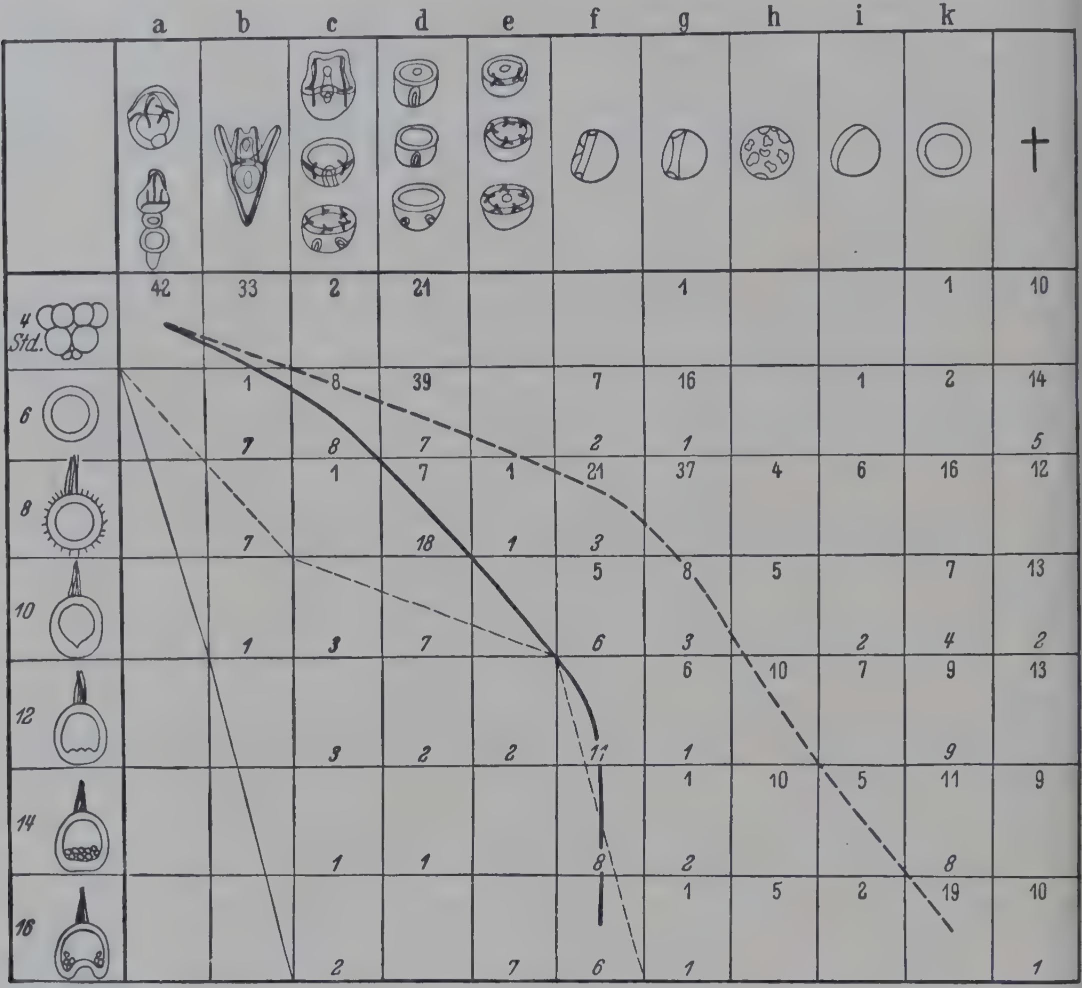


Fig. 277. Plot of the course of animal pole determination in the echinoderm embryo, lith being used as the vegetalising influence (see text).

eventually to a perfect pluteus (O). It was thus proved that under the influe of lithium, the most vegetal material of the animal half had become as veg as any micromeres.* Left to itself, the animal half after the lithium treatm would have given a not very well-formed pluteus (L), but this, nevertheless far more vegetal than anything the animal half could form by itself.

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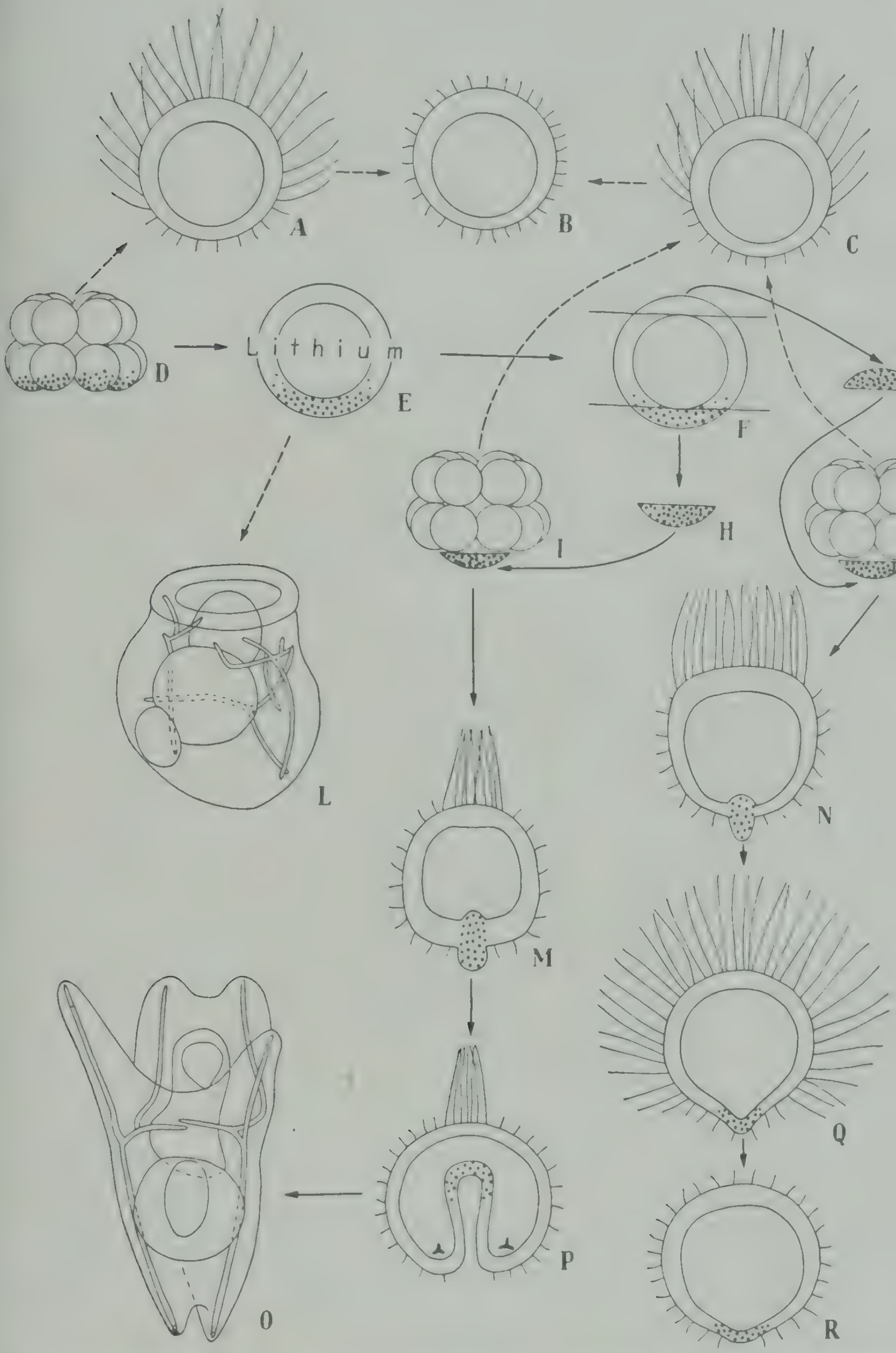
* There is a parallel here with the awakening of induction power when a piece of presump ectoderm is allowed to remain for a time in the amphibian organisation centre (see p. 156). was well realised by Hörstadius[5], but he unfortunately referred to such an activated piece of ti as a "secondary organiser"; from p. 290 it will be seen that this terminology invites confu and should not be adopted. A closer analogy would be the activation of a piece of presump ectoderm by boiling or by treatment with dyes (see p. 189).

That this remarkable effect is not due to the mere transportation of a certain mount of Li′ into the animal half at (I) was proved in a subsidiary experiment



. 278. Demonstration of the vegetalising power of animal fragments themselves vegetalised by lithium (see text).

which not the most vegetal material of the lithium half-embryo, but the most imal material, was taken, stained vitally (G), and attached to an animal half the same vegetal position as before (K). Only the slightest vegetalisation resulted from this (N), and later on there was no invagination (Q), and no formatio a pluteus (R). The conclusion must therefore be that Li′, in vegetalising animal half placed in it, begins by enormously raising the "potential" of vegetal pole of the half-embryo, at least as high, in fact, as that of the microm themselves, but that the animal pole of the half-embryo still retains its autono as indeed it must if any regulative approach towards the normal pluteus is t obtained. We shall willingly agree with Dalcq[7] that "this experiment, with accurate performance of the various necessary controls, is one of the finest techn achievements in causal embryology".

Many other similar experiments have been made. For example, s fragments of an animal half, treated with lithium, will, when implanted i the blastocoele cavity of an animal blastula, otherwise doomed to rem so, induce there the full invagination of the archenteron (Hörstadius[6]). A animal halves, treated with lithium, will gastrulate and end in perfect pl (v. Ubisch[2]).

2·95. A critique of the chemical aspects of echinoderm determinati

We have now to add some further facts on the biochemical side. Since vegetalising action of Li′ had been found to be intensified by respiratory inhibit such as CO and KCN, it was natural to expect to find catalysers of respirat on the other side. This was fulfilled when pyocyanin was found by Runnströ to have a slight antagonistic effect on the morphological action of lithium, a later Runnström & Thörnblom found that the animalising action of SCN′ greatly intensified and accelerated in the presence of pyocyanin. In 0·03 iodoacetate traces of vegetalisation (poor development of stomodaeum and arms) were seen by Runnström[17]; this was stopped if 0·02 M pyruvate was add In 0·0003 M bromacetate Tchakhotin[3] observed a great proliferation of prim mesenchyme, so as to fill the blastocoele cavity; this presumably counts a vegetalisation, if an anomalous one. The systematic experiments of Lindahl Stordahl showed that SO_4''-lack* (strongly animalising) has no morphological deleterious effect upon animal halves, nor upon larvae previously animalised SCN′. On the other hand, animal halves treated with Li′ suffered severely wh placed in sulphate-free sea-water and did not show the vegetalisation (formation, etc.) which they would have done in sea-water containing sulpha Vegetal halves were also more severely affected by SO_4''-lack than whole embry they produced a variety of forms and showed a tendency to degenerate. A dir test of the effect of indoxyl sulphate gave indications of a strong animalis action if the conditions were favourable.

Still pursuing the idea of inhibition of metabolism and development of aroma substances, Lindahl & Stordahl carried further the well-known observation Peebles that during the development of echinoderm eggs some substance is gi ff which inhibits the development of other eggs.* They found that in the absence f sulphate more of this inhibitory substance is given off than in its presence tatistical experiments on otherwise normal embryos). Attempts to trace it ectroscopically have not so far succeeded. From the results on animal and egetal halves just described, Lindahl & Stordahl concluded that the site of rmation of the inhibitory substance is the presumptive endoderm and adjacent egetal regions.

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* Among the minor effects of vegetalisation by SO_4''-lack is the failure of the red pigmen appear in the egg (Lindahl & Örström[1]), and its formation can be intensified by excess of sulph A direct connection is difficult to believe, for echinochrome, though at one time thought to con sulphur, is, according to the latest analyses, sulphur-free (Lederer & Glaser).

The kinetics of Li′ inhibition were further studied by Lindahl & Öhman. ithium added at later stages, after a certain amount of the "rising" fraction of spiration has gone on (see Fig. 275), will not inhibit this fraction, but only rther increases of it. Hence the effect on the respiration is smaller the later e lithium is applied, and this exactly parallels the morphological effect. On e other hand a remarkable dissociation between the respiratory and morpho- gical effects occurs with rising temperature. At first (between 20 and 24°) both e intensified, and the increase in the "rising" lithium-sensitive fraction of spiration is relatively greater than that in the lithium-insensitive part, but at ° some new limiting factor comes into play, for the morphological effect is ronger than ever, but the inhibition of respiration disappears. This suggests at the respiratory inhibition is not an indispensable link in the chain of causes ading to vegetalisation. Moreover, the morphological effect begins before the spiratory inhibition is detectable.

Lindahl's earlier experiments[7] on cytolysed egg-breis were carried further by indahl & Öhman. Runnström[12] had shown that hexose-monophosphate and xose-diphosphate would act as hydrogen-donators to methylene blue with g-brei in anaerobic experiments. He had also demonstrated the presence of -zymase in sea-urchin eggs. In Lindahl's experiments, the conditions had lmittedly to be rather carefully chosen in order to demonstrate any inhibitory fect of Li′. Using only the donators present in the egg-brei itself, Li′ had even acceleratory effect on methylene blue reduction. On centrifuging, however, se auto-donators could be more or less completely removed, and then an ibitory effect of Li′ at 0·256 N LiCl could be shown, with hexose-mono- sphate as donator. But the effect was not very large, and excess Na′ and K′ wed something of the same kind. Both Li′ and K′ activated when dilute l inhibited when concentrated, but the inhibitory effect of the former began ore that of the latter. Lindahl & Öhman now put together egg-brei, hexose- hosphate, co-zymase, and pyocyanin, obtaining a not very large respiration c.mm. 1·5 c.c. egg-brei/75 min.). With Li′ this was inhibited, but at the ghest concentration used (0·135 M) only 24 %.

Pyocyanin added to respiring eggs accelerated the constant fraction of respira- n but not the "rising", lithium-sensitive one. This suggests that the oxygen- tivating systems and not the dehydrogenases are the limiting factor in it.

It must be remembered that Peebles also found stimulatory substances given off, which may lain the slightly faster rate of cleavage of echinoderm eggs in crowded cultures than in sparse s, persistently claimed by Allee & Evans. Cf. also the work of A. R. Moore[7] on cleavage- ulating substances within the eggs (p. 364). It is to be feared that both stimulatory and ibitory substances are given off, and their identification will not be an easy matter.

Urethane, on the other hand, though inhibiting development and respira as in the classical experiments (*CE*, p. 625), never upset the gradient sys so that plutei later formed were morphologically normal, even when the uret concentration was so chosen as to imitate the degree of respiratory inhib shown by a strong Li′ concentration. Hypotonic and hypertonic sea-water inhibit respiration (Borei[2]), but they have no effect on the gradient system.

To what has already been said regarding the gradients between the animal vegetal poles we need only add that if the distance between them is artific lengthened, the influence takes longer to operate. Lindahl[3] accomplished thi forcing eggs into a glass capillary tube smaller than their normal diamete certain cases the longitudinal axis of the "sausage" egg was identical with animal-vegetal axis. As a result the animal pole of the embryo was r animalised than it should have been (hypertrophy of ciliary tuft, displacem of stomodaeum vegetally, etc.). Hörstadius[10] accomplished it by beautiful c striction experiments, in which he succeeded in ligaturing sea-urchin emb

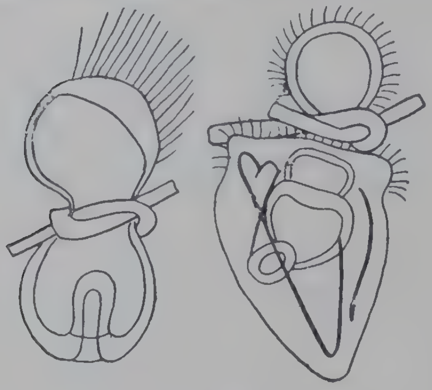

Fig. 279.

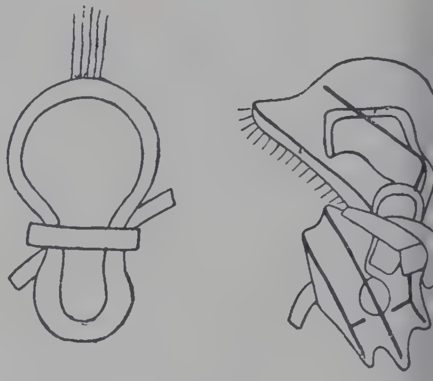

Fig. 280.

Fig. 279. Effect of a tight equatorial ligature made at the 16-cell stage (see text).

Fig. 280. Effect of a loose equatorial ligature made at the 16-cell stage and tightened at th blastula stage (see text).

at early stages with fibres of silk. A tight equatorial constriction in the 16- stage led to animalisation of the animal half, and attempted normal developm of the vegetal. A loose equatorial constriction in the 16-cell stage, followed by tightening of the knot in the late blastula stage, gave development of the an half more in accordance with its prospective significance (see Figs. 279 and 2 All these experiments demonstrate well enough the diffusion of some subst through the embryo, or at least the passage of some change. They are the coun part of the constriction experiments made on the eggs of amphibia and ins (see pp. 103 and 459), but few would have thought it possible that they c be performed on so minute and fragile a sphere as the developing echinod egg.

Viewing critically the mass of work of the Scandinavian school on echinod development, we find that it hangs together remarkably well. That there are centres of morphogenetic influence and a gradient system which re-forms a certain experimental interferences must be regarded as established. It is deniable also that the actions of these centres may be imitated by a variet ıemical agencies, and it is at least exceedingly probable that the two centres e characterised by two different kinds of metabolism. That they produce each definite morphogenetic substance or substances is likely. But with the statement that the animal pole is associated with carbohydrate catabolism and the ·getal pole with that of protein, we reach the point at which caution is necessary. he carbohydrate attribution rests mainly on the rather unsatisfactory experiments ith hexose-phosphates in breis of cytolysed eggs (Runnström[12]; Lindahl[7]; ndahl & Öhman); the vegetalisations so far produced by the halogen acetates ve been too weak or anomalous to give it strong support. Moreover, Needham Needham[5] were unable to find any trace of vegetalisation in echinoderm ıbryos cultured in solutions of *dl*-glyceraldehyde of strength amply sufficient inhibit glycolysis. Further experiments will be necessary before convincing ·oof can be said to have been provided that animal-pole metabolism is carbo·drate breakdown, and it is not too much to hope that with modern methods rect R.Q. measurements on animal and vegetal halves will become possible. milarly, the appearance of aromatic breakdown-products is really no proof that e catabolism of vegetal regions is predominantly that of protein. Protein eakdown might simply take a different course there. Here again, direct timations of ammonia excretion should help to clarify the issue (cf. p. 626).

Another important question is the degree of specificity of the vegetalising ents. Exo-gastrulation has been produced by a number of salts (e.g. of K', ı′, Hg'', Ca'', etc.) by McArthur and by Waterman[1], but exo-gastrulation, as ndahl[7] points out, need not imply vegetalisation, if the proportions of material ıld exactly to their prospective significances.* Thus Lindahl accepts as getalisation only the results of Waterman with Mg''. On the other hand, he ;o accepts the vegetalisations obtained by Motomura[1] using auxin fractions, ·cogen, and even $KClO_3$. Such unspecificity calls for further investigation.†

There is no space here to go into the question of the nature of the action of ’, but it remains highly obscure. That it actually penetrates into the egg we ıw from the spectrographic measurements of Ranzi & Falkenheim on the ash ithium larvae. One clue was provided by Runnström[5], who in connection with examination of the egg from a colloidal point of view (Runnström[4]; Lindahl)[1], died it under dark-ground illumination. After fertilisation there arises an nge-yellow ring or fringe, probably of lipoidal nature, and occupying approxitely the position of veg_2; during gastrulation this enters at the blastopore when endoderm is forming by invagination (Fig. 281). In embryos treated with ıium, however, this ring of material extends much farther up the side of the trula (Fig. 281), and perhaps is not wholly invaginated. Other clues have been ıght for in biochemical experiments on other material. Thus the respiration, ·mentation, and hydrolysis of hexose-phosphate by yeast may under certain conditions be inhibited by Li′ (Lindahl[6]; Boas). But others have found influence on yeast proliferation or fermentation (Lasnitzki & Szorenyi) nor the $Q_{\mathrm{L}}^{\mathrm{N}_2}$ of tumour tissue (Lasnitzki[2]) nor on Q_{O_2} of various animal tissues (Kisc It may indeed be said that up to the present time a biochemical effect of lithi equivalent in magnitude to its morphological effect has been sought for in v Turning, therefore, to more biophysical effects, Spek[1] maintained that the prim effect of Li′ must be a swelling and precipitation of the outside of the veg cells, and it is almost certain that physical action of this kind must play a p in the Li′ effect. Runnström[15], examining *Arbacia* and *Echinarachnius* blastu with micro-dissection needles, found that after treatment with lithium, physical state of the cells was quite different. Cells of normal blastulae, t away from their neighbours in the ectoderm, immediately became spherical, those from lithium blastulae retained for a long time a spindle-shaped appearan The hyaline plasma layer, however, is softened, not hardened, by lithium.

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* Γhis would presumably rule out the mechanically produced exo-gastrulae of I. Törö, and the ırium and uranium malformations of Okada[1].

† :hild[7] even relates that tobacco smoke, kept above the cultures, will cause exo-gastrulation. ·ould be interesting to know whether the Scandinavian school would accept such exo-gastrulae howing true vegetalisation. Nicotine in minute concentrations does inhibit certain enzymes. ɔacco ash is said to be rich in lithium.

Equally obscure is the mechanism of the intensification of the Li′ effect CO and KCN. It has long been known that CO to some extent *accelerates* (inhibits) the respiration of unfertilised echinoderm eggs (Runnström[7]), t respiration of insect embryos during the diapause (Bodine & Boell[1] for *Melanopl* Wolsky[1] for *Bombyx*), and the respiration of yeast with formate as substr (Örström[2]). Similarly, CN′ accelerates (not inhibits) the pyocyanin-raised respi tion of unfertilised echinoderm eggs (Runnström[14]), the respiration of certa mammalian tissues (Kisch[2]), and the respiration of yeast with dioxyacetone substrate (Lindahl & Örström[2]). At low concentrations, therefore, the accelerato effects of CO and KCN may outweigh the inhibitory ones. Dehydrogenas too, may sometimes be activated, not inhibited, by KCN (Leloir & Dixo Lindahl & Öhman consider, therefore, that the formation of some kind of compl with heavy metals previously present, themselves the inhibitors of sulphydr catalysed reactions, for example, is likely to be the explanation of these effects KCN and CO.

It must be concluded that echinoderm development offers special attractio for the study of the relations between metabolism and determination.

The gradient hypothesis, as we have seen, has proved not unfruitful in t experimental morphology of echinoderms. But if it had T. Boveri for its fath C. M. Child has been its prophet. Among his earlier investigations were sor in which the disintegration of echinoderm embryos in toxic solutions (KC ammonia, etc.) was said to begin from the animal pole in the cleavage, blastu gastrula, or pluteus stages, and to progress from there (Child[2]). Recently has studied the question again, using the much more acceptable technique differential anaerobic reduction of vital dyes (Child[6]). With methylene blue janus green reduction occurs first at the animal pole in the cleavage stages a the blastula. As soon as the micromeres have migrated into the blastocoele cavi they constitute a second, vegetal, centre of reduction activity. At gastrulati reduction starts simultaneously at the animal pole and the tip of the endoderm invagination, spreading everywhere from these two points. As gastrulati proceeds, the region of rapid decolorisation moves excentrically from the previo

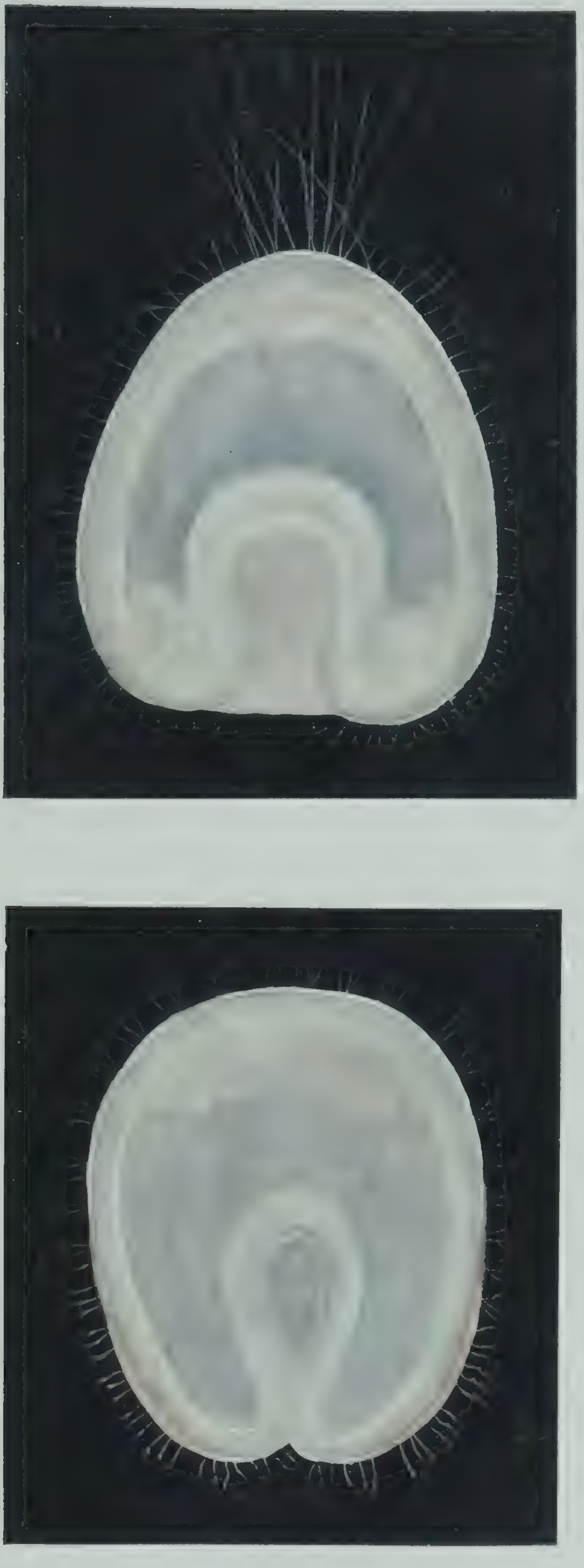

Fig. 281. The orange-yellow fringe at veg_2 in the echinoderm gastrula.

imal pole to meet the endoderm at the point where the stomodaeum will form. lithium exo-gastrulae (Child[7]) the reduction seems to begin at the junction tween the ectoderm and the endoderm, spreading outwards in both directions, d sometimes the animal pole reduction centre disappears altogether. These eresting results agree more nearly with the views of the Scandinavian school an one would think from Child's discussion of them.*

Work along the same lines was continued on lithium larvae by Ranzi & lkenheim, using a variety of reducible dyes, with substantially the same results; animal pole becomes less highly reducing. The indophenol blue reaction, α-naphthol peroxidase reaction, and the nitroprusside test for fixed –SH, wever, varied little as between control and lithium larvae (cf. p. 136). But otti[3] was able to observe definite shifts in the benzidine-peroxidase responding a (characteristic of presumptive endoderm and mesoderm), lithium extending and thiocyanate restricting it.

2·96. Later determinations

Up to the present we have spoken only of the animal-vegetal gradient system. t this is primarily of larval significance. Ultimately the dorso-ventral axis is re important, since most adult echinoderms possess a radial symmetry. We not yet know what determines it; as may be seen from the review of Hörstadius[6],

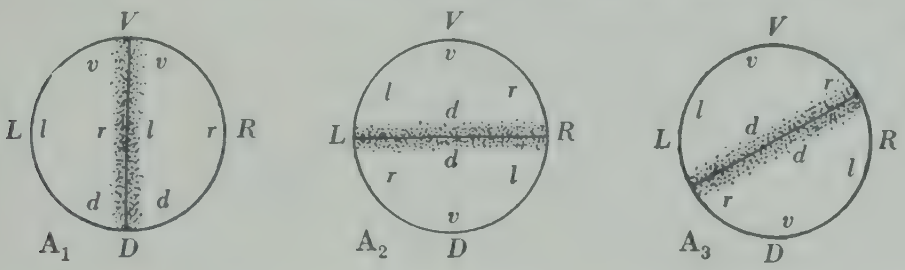

Fig. 282. Determination of the dorso-ventral axis in half-embryos (see text).

elation to the point of entry of the spermatozoon or the first cleavage plane, nstance, is still an open question. It is certainly much less stable than the al-vegetal axis, for it may be affected by centrifugal force (Runnström[2]; dahl[4]; Pease[1]), and when Lindahl[3] forced eggs through a capillary tube, as dy described, producing "sausage" embryos, the front end of the sausage always afterwards the ventral pole. If heavy vital staining is applied to it, ever, this relation can be reversed, and it will become the dorsal pole.

he determination of the dorso-ventral axis in half-embryos presents the arkable feature that it often involves a complete inversion of the previous rity (Hörstadius[6]; Hörstadius & Wolsky). If the cut is meridional, separating t and left halves (see Fig. 282), the polarity remains as it was, but if the cut quatorial or oblique the subsequent dorsal pole always forms at the cut surface, hat the dorsal half has its polarity inverted. A pleasing, if speculative,

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her organisms, invertebrates, protozoa, etc., have been investigated with the dye-reduction od, with results more or less illuminating (Child[5]; Child & Watanabe; Watanabe; Child & n). Cf. also the similar work on the amphibian gastrula (p. 203) and the chick blastoderm 35).

explanation for this has been put forward by Bernstein[2] (see Fig. 283). Suppo there to be a ventral pole substance, and supposing this to be water-solu it would be expected to diffuse away from the cut surface. In this case gradient of it would be reversed in the case of the dorsal half but only steepe in the case of the ventral half. By the time the late blastula stage is reach inversion of the dorso-ventral axis becomes impossible. That the ventral p naturally determined, is characterised by metabolic peculiarities appeared fr the work of Förster & Örström, in which the larger of two depressions forr when fertilised but uncleaved eggs are treated with KCN always occurred at a point which vital staining showed later became the ventral pole. This observation was later much extended by Pease[2], who found that on exposure of the egg to a chemical diffusion field, the exposed (i.e. most affected) side always became the dorsal pole. The effective substances were, however, too miscellaneous to allow of any definite biochemical implications.*

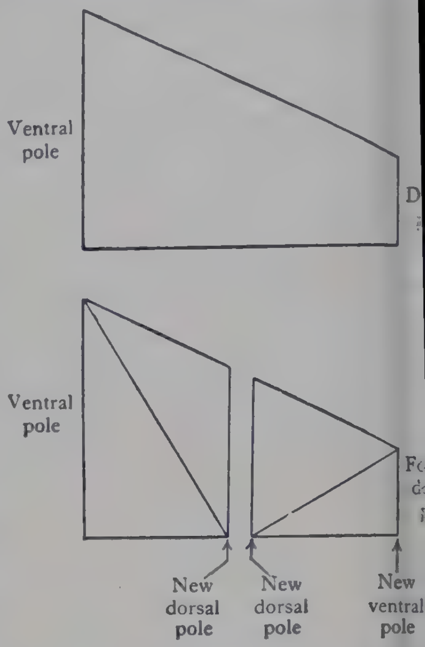

Fig. 283. Bernstein's hypothesis plaining the reversal of do ventral polarity in half-embryo

In later development, many of the echinoderms undergo a metamorphosis as wonderful as any to be found in the animal kingdom. The details of how the echinus-rudiment, for example, is formed within the body of the pluteus, the radially symmetrical adult converting into itself the whole substance of the bilaterally symmetrical pluteus larva, must of course be found in the classical descriptions (such as that of McBride[2]). But as it is lik that important induction processes go on during this metamorphosis, a b account of what happens is necessary. The coelom begins as a diverticul from the archenteron, budded off at its tip, near the stomodaeum, and this b presently splits into two (cf. Fig. 284 A). The left and right coeloms rem applied closely to the gut and stomach but in time become constricted i anterior and posterior portions (Fig. 284 B). Later, at the echinopluteus st (see Fig. 284 C, D), the left anterior coelom buds off a vesicle called the hyd coele, which will later give rise to the adult water-vascular system. The n by which it remains connected to the coelom will eventually become the st canal of the adult. Now between two of the larval arms, over the hydroc as it approaches the ectoderm, an ectodermal invagination occurs, the amnic invagination (cf. Fig. 284 C), which before long is cut off from the exterior a becomes a cavity (Fig. 284 F). The hydrocoele soon changes to an annu (Fig. 284 E), and through the centre of this ring, with the amniotic cavity ab it and the coelom below, runs the axis of radial symmetry of the future discoi sea-urchin. The whole is termed the echinus-rudiment. For the present purp we need not follow its further development.

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* Effective: CN', $Fe(CN)_6'''$, I', $CH_2I.COO'$, picric acid, urethane and 2:4-dinitrophe ineffective: F', AsO_3', Cu'', $H_3P_2O_7'$ and malonate.

The most important point here is that the adult structure arises in normal velopment only on the left side, not on the right. However, the literature ntains a number of descriptions of larvae containing two echinus-rudiments, e on each side, and McBride's observations[1] on such rare naturally occurring rvae in 1911 led him to try to produce them experimentally. This he was le to do (McBride[3]) by the use of hypertonic sea-water, but not in large antity. It was at any rate clear that competence exists on the right side of the

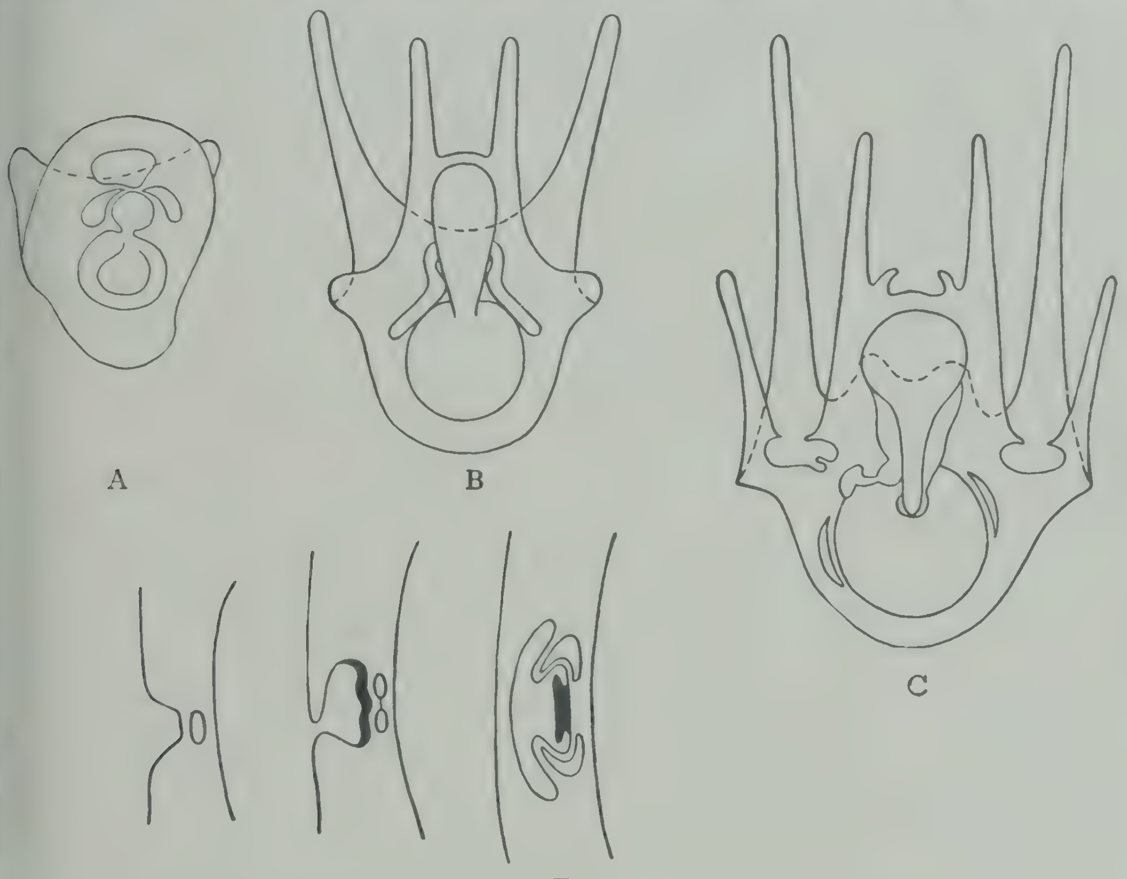

Fig. 284. Sketch of echinoderm metamorphosis (see text).

a. Noticing, however, that wherever a hydrocoele appeared, there also eared the amniotic invagination and other later transformations of the ecto- m, such as the formation of the spines, he concluded that the hydrocoele bably gave out a morphogenetic hormone and that the ectodermal changes e a dependent differentiation.* “We can have”, he said, “a right hydrocoele hout a right amniotic invagination and right dental sacs” (developed from the terior coelom), “but we cannot have these latter structures without a right drocoele.” This conclusion was later somewhat weakened by observations

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IcBride himself compared the induction of the amniotic invagination in echinoderm metamor- sis with the induction of the lens by the amphibian eye-cup. It is interesting that he wrote e body of an embryo is not...a mosaic of pieces each destined to form a particular organ, but sists of sheets of indifferent material, without form and void, on which a formative ‘something’ ks and *evokes* the beautiful detail of the adult structure.” And he spoke of this something hormone. How enlightened his general position in embryological theory was in this earlier od may be seen by a glance at pp. 528, 529 of his *Textbook of Invertebrate Embryology* (1914). ortunately, it did not remain so[4].

which show that a slight degree of amniotic invagination may be found with the action of a hydrocoele (Runnström[1]; Runnström & Runnström). But th is general agreement that its development cannot proceed further than the sligh initial stages without the underlying structure (cf. the induction of the oral a in the pluteus, p. 480 above).*

The Echinopluteus, difficult to culture in the laboratory, has not lent itsel transplantation experiments. Hörstadius[6], however, describes the behaviou right and left blastula halves cultivated up to metamorphosis. Whereas left hal always produced left hydrocoeles as normally, right ones were erratic, giv hydrocoeles on either side or on both. The meaning of this is not understoo

The exact form of the spicules of the skeleton varies as between differ species, and it has therefore been possible, by implanting the micromeres of species into the blastocoele of another, to carry out xenoplastic experiments test the relative part played by nucleus and cytoplasm when deriving fr different species (cf. p. 362). This work is summarised in the review v. Ubisch[6], who believes that the cytoplasm has no influence on skeletal form, Hörstadius[7] is of the opinion that this line of work is not as yet decisive.

2·97. Organiser phenomena in echinoderms

We come lastly to a much canvassed question, which is yet perhaps not important as it has sometimes seemed, namely the homologising of the cent in echinoderm development with those in that of vertebrates.† Hörstadius[3] at first content to call the vegetal pole the organisation centre, but later recogni the status of the animal pole as another organisation centre.‡ All observers agreed, however, that the vegetal pole is, in a sense, predominant, and sho the most powerful induction effects. Induction in early sea-urchin developm is, of course, rather different from amphibian induction. Micromeres definit induce archenteron invagination. They also induce other forms of vegetalisati and in those cases where they alter the presumptive significance of adjac material, they act just as does the transplanted dorsal lip of the blastopore. the formation of many organs (such as the stomodaeum and the band of ci will not take place if the embryo is too animal or too vegetal; only if there proper balance between the two centres. Hence if, for instance, too ma micromeres are put into an animal half, these structures, which would form un balanced conditions, no longer do so because the embryo is now too vegetalis Again, when the micromeres are transplanted to the animal pole, an archente ay be induced there, but the archenteron at the other pole forms just as well ecause the most vegetal material takes on the characteristics of the vegetal pole. new vegetal organiser is in fact formed.

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* If either the hydrocoele or the amniotic invagination fails to develop properly, the en normally used in further metamorphosis will run to the formation of many queer structures n otherwise seen (cf. the description of Runnström[1]).

† It may not be irrelevant, in view of the similarity between the embryos of echinoderms vertebrates as regards their late determination and hence their immense powers of regulatio refer to the views of Bateson on the evolutionary connection between these two phyla, so prisingly confirmed in biochemical experiments by Needham, Needham, Baldwin & Yudkin Needham & Needham[4]) and many others.

‡ Spemann lent his authority to this view in the omitted eighteenth chapter of his book "Man könnte die Sache vielleicht auch so ausdrücken, dass beim Seeigelkeim zwei gegeneinan gerichtete und ineinander greifende Induktionsströme wirksam sind" (p. 273).

One disadvantage of the echinoderm material as compared with the amphibian that so far it has proved impossible to test the effects of implantation of material lled in different ways, or of chemical extracts or substances. This is only rtially compensated for by the remarkable instability of the echinoderm gradient stem towards external ionic factors. Nevertheless, the Scandinavian hypothesis specific morphogenetic substances, formed at the animal and the vegetal poles spectively, and associated with two different kinds of metabolism, remains the ost satisfactory one for future work.

It is not easy to regard the animal-vegetal gradient of the echinoderm egg analogous to the animal-vegetal yolk adient of the amphibian egg (*A–B* in g. 285), for no active centre has been und at the vegetal pole of the latter, less we were to think of the as yet identified site of endo-mesodermal deter-ination referred to above (p. 163) in con-ction with the determination of endo-rmal organs (*E*). It is not quite clear ether this is in the mind of those (Dalcq Pasteels; Dalcq[7], p. 147) who identify the luence radiating from the vegetal pole of e echinoderm egg with the yolk gradient the amphibian egg (cf. p. 220). The same thors regard the influence radiating from e animal pole of the echinoderm egg with grey-crescent field of the amphibian egg p. 220). As there is no neural induction in the formation of the pluteus, se identifications leave a feeling of uneasiness in the mind.

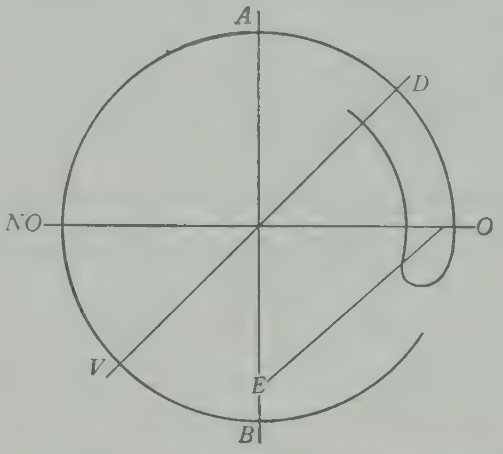

Fig. 285. Diagram of the amphibian gastrula. *A–B*, animal-vegetal yolk gradient; *O–NO*, “organiser-non-organiser” gradient; grey-crescent field; *E*, hypothetical origin of endodermal determination; *D–V*, dorsal-ventral gradient.

indahl, on the other hand ([7], p. 355), suggests that the ventral pole, account of its importance for bilateral symmetry, should be regarded as esponding to the organiser. He points out that the induction of a secondary ryo by the organiser in amphibia means a doubling of the dorso-ventral axis *V*), while the induction of an archenteron in echinoderms by vegetal material ns a doubling of the egg axis (*A–B*). Doubling of the dorso-ventral axis can ourse occur also in echinoderms. On this view there would be nothing in phibia corresponding to the animal and vegetal centres.

s against Lindahl, however, Hörstadius ([6], p. 166; [10], p. 236) reminds us t the position of the ventral centre is very unstable, that it has not been wn to have any inducing power, and that there is also the dextro-sinistral to be considered. This is not without importance in view of the later asym-ric origin of the echinus-rudiment in metamorphosis. Hörstadius further s attention to certain analogies between the dorso-ventral axis in the amphibian gastrula (or the "Organiser-non-organiser" axis of v. Ubisch[5]; *O–* and the animal-vegetal axis in echinoderms. Dorsal lip region, isolated, as we have seen (p. 283), give much more differentiation than its prospec significance would allow. Then a piece of dorsal lip transplanted to the sumptive neural region will turn into neural tube, whereas a piece of presump neural ectoderm transplanted to the dorsal lip region will act as organiser become mesoderm after invagination (p. 170). The former phenomenon may akin to the suppression of the vegetal centre in an animalisation. The latter be akin to the suppression of animal differentiation by vegetalisation. Just a the case of the amphibia these experiments are most easily explained by liberation or the failure of liberation of an active morphogenetic substance f inactive combination, so when the gradient re-forms in the echinoderm egg a removal of animal or vegetal material, a new liberation of substances may supposed to take place. This is much the same as the reversible reactions formation assumed by Lindahl[7]. During such re-formation, we cannot a postulating, as v. Ubisch rightly says[5], some kind of *Entmischung* (re-sor out of molecules) of the morphogenetic substances. In this process of establishment, the formation and destruction of "masking complexes" v active proteins may play an important part.

Lastly, it may plausibly be held that the echinoderm animal-vegetal gradi system is, as it were, entirely enclosed in the amphibian organisation cen since this centre is the carrier of individuation.

Part Three

THE MORPHOGENETIC MECHANISMS

THE MORPHOGENETIC MECHANISMS

3·05. Introduction

In the development of an animal embryo, proceeding normally under optimum onditions, the fundamental mechanisms constitute a perfectly integrated whole. hey fit in with each other in such a way that the final product comes into being y means of a precise co-operation of reactions and events. But it seems to be very important, if perhaps insufficiently appreciated, fact, that these fundaental mechanisms are not separable only in thought; that, on the contrary, they an be dissociated experimentally or thrown out of gear with one another. This onception of gearing still lacks a satisfactory name, but in the absence of better ords, *Dissociability* or disengagement will be used in what follows. It is already lear that embryonic growth can be stopped without abolishing embryonic spiration, and conversely, it is possible that growth or differentiation, under ertain conditions, may proceed in the absence of the normal respiratory proesses. There are many instances, again, where growth and differentiation are parable. It is as if either of these processes can be thrown out of gear at will, that although the mechanisms are still intact, one or the other of them is cting as "layshaft" or, in engineering terms, is "idling".

Since one of the principal aims of embryology must be to understand the eans whereby in the normal embryo the fundamental mechanisms are integrated, is probably worth while to devote some attention to the cases in which this tegration is artificially broken down. We shall then discuss in succession the *rowth-process* itself in its chemical bearings, the various forms of *respiration* and *etabolism*, and the hints which we possess about the way in which *polarity* and *nmetry* properties of embryos arise. All this is involved in the mechanism of rphogenesis.

3·1. Dissociability

Let us first define more clearly the fundamental processes which are under nsideration. For the present purpose, the rough classification shown in Table 28 ll be adopted.

3·11. Disengagement of growth and differentiation

A certain incompatibility between these two great processes is very widely en. It is a commonplace that the higher the differentiation of a tissue the fewer ll-divisions take place in it, and conversely. Or a myxomycete plasmodium, for stance (see p. 672), grows chaotically as long as it is fully nourished, but when nditions deteriorate it goes into differentiation and produces beautifully rmed fruits. Some authors, such as v. Bertalanffy[3], have urged the existence a causal relationship between rising differentiation and falling specific growthte.

Table 28. *Fundamental processes in morphogenesis*

A. Growth: increase in spatial dimensions and in weight	1. *Multiplicative*: increase in number of nuclei)
	2. *Multiplicative*: increase in number of cells
	3. *Auxetic*: increase of size of cells
	4. *Accretionary*: increase in amount of non-living structural matter
B. Differentiation: increase in complexity and organisation	1. Increase in number of kinds of cells: (*a*) invisible. *Determination* of fates; gain or loss of *Competences*, etc. (*b*) visible. *Histogenesis*
	2. Increase in morphological heterogeneity; the assumption of form and pattern; *Organogenesis*: (*a*) *individuative*: due to the primary individuation field of the organism before any of its parts have become functional (*b*) *corporative*: due to the mutual interactions of functional parts
C. Metabolism: the chemical changes in the organism	1. *Respirative*, i.e. oxidation of carbon compounds
	2. Fermentation or *Glycolysis*: the non-oxidative catabolism of carbohydrate
	3. *Catabolism of protein*
	4. *Catabolism of fat*
	5. *Characteristic chemical activity*: e.g., pigment formation, or the synthesis of glycogen

Notes to Table 28

(1) The grouping of the processes is difficult and perhaps necessarily arbitrary. Thus, might possibly be placed in B; A4 in C; and A1 and A2 in B.

Although increase in number of nuclei and increase in number of cells is not growth in sense of size expansion, it is so intimately bound up with growth that it is placed in group Nuclear and cell division can occur without size expansion in many organisms, e.g. developi marine invertebrates in the cleavage stages, but here respiring protoplasm grows at the exper of inert yolk.

(2) *Multiplicative* is equivalent to the *Meristic* of the botanists.

(3) B1 needs further comment:

(i) Increase in number of kinds of cells may be either reversible or non-reversible. T specialisation of the histological elements in a tissue such as the kidney (which may be revers in rapidly growing explants) is reversible (*Modulation*), but the determinations of early develo ment are generally not reversible. Thus the kidney cells can lose their visible differentiatio but they cannot differentiate off into liver cells.

(ii) The notion of *Competence* has been discussed already (pp. 112 ff.).

(iii) Huxley[9] has introduced two further terms—"Histodifferentiation" and "Auxar differentiation". He distinguishes two periods in the growth of a part, firstly, a laying down general form, accompanied by rapid morphological and histological change; secondly, a cessati of histological change, and a reduction of the form change to "quantitative alterations in proportions of the definitive structural plan". For these two periods he suggests the ter "Histodifferentiation" and "Auxanodifferentiation" respectively. I am unable to see in wh way this latter process differs from Growth, since growth, defined as increase in spatial dimensio need not necessarily take place equally in all of them. The valuable investigations of Huxley heterauxesis show, indeed, that it hardly ever does. And reference may be made to those direct cell streams, the existence of which is so important for early differentiation, and the nature which is so obscure (see pp. 145 ff.). Again, if we define differentiation (as in B) as increase complexity and organisation, "quantitative alterations in proportions" of a fixed plan can harc be included in it. Such alterations amount to deformations and appertain rather to the sphe of relative Growth. "Histodifferentiation" appears to be covered by B1(*b*) and B2(*a*).

(iv) B1(*b*) is termed by Woodger[1] "Elaboration". "Differentiation" he defines as "a proc whereby two (or more) different parts come into being by the separation unequally between th of something previously present in one part (or whole)."

(4) B2(*a*) is given the term *Individuative* after the concept of the individuation field, d cussed on pp. 125 ff.

B2(*b*) is given the term *Corporative*, since in the late stages of development (the seconda or functional period) the various parts of the organism react upon one another as members a corporation.

(5) It may be noted that processes C1–4 are all energy-providing processes, whereas C5 ne not be, and may be the reverse.

3·111. Growth without differentiation

Growth without differentiation is likely to occur whenever there is any failure the formation or liberation of primary or secondary organisers. In a preceding ection (2·371) we have seen many instances of this occurrence, especially the anidian" embryos of sauropsida in which the primary organiser is suppressed. irst discovered by Panum in 1860, such cases are referred to here only to nphasise their logical position. This they have in common with malignant mours, already discussed in Section 2·38. In connection with this, it is of terest that in birds normal growth of the yolk-sac (Aliberti; Remotti[4]), the lantois (Katzenstein) and the amnion (E. Wolff[1]), will go on after the death the embryo itself.

In one sense, all developmental arrests belong in this section. There are a great umber of well-known malformations due to the failure of some morphogenetic ocess (e.g. hare-lip in mammals). Another instance of this, discussed by udger and Kyle, is the occasional failure of flat fishes to turn over, thus taining their bilateral symmetry throughout their lives.

3·112. Differentiation without growth

Another instance of the disengagement of growth and differentiation is afforded the work of Hoadley[3], who transplanted eye-cups of the chick embryo to the orio-allantoic membrane and allowed them to develop there until they had ached a degree of differentiation equivalent to that of the controls. Then by aking and weighing wax models he ascertained the relative weights, and always und that the controls were much heavier. But there was a direct relation with e, for the younger the transplant at the time of transplantation, the smaller the entual fully differentiated organ;

At the time of transplantation		After 8 days
Embryo age (hours)	No. of somites	size of control : size of transplant (1 : x)
48	28	1 : 0·219
35	14	1 : 0·098
20	0	1 : 0·0136
4	0	1 : 0·00075

us, in all cases the growth of the graft was very much inferior to that of the trol in its normal situation, although differentiation, as judged by the degree istological development and cellular elaboration, was the same. There seems obvious mechanical reason why this suppression of growth should occur, for transplanted fragment becomes surrounded by highly vascular tissue between epithelial margin of the allantois and the repaired chorionic epithelium. pillaries of the host membrane ramify freely in the neighbourhood of the nsplant, the cells of which should have no difficulty in obtaining from them nutritive building stones required for growth.

Ioadley explains these results by the assumption of a lag period immediately er transplantation which would be felt more acutely by the youngest and refore smallest fragments. During this time cell-division would be suppressed, but histogenesis would be proceeding. It remains obscure, however, why cell-division should be more fully suppressed in the case of the youngest gr

“Differentiation”, says Hoadley[3], “is not primarily dependent upon mechanism involved in specific cell-divisions, but to a large extent takes p independently of these. Inasmuch as this is true, and inasmuch as ty development depends not only on the subordinate differentiation of the stituent parts of the embryo, but also on the spatial relations between them their size, the truth of the following statement is evident—‘typical develop is the result of the usual balance between morphogenetic (form producing) histogenetic (cell differentiating) processes’.” The derangement of this bal appears again in other instances. Thus Waddington[1], in the course of his exten studies on the chick organiser *in vitro*, observed that the mere developmen the blastoderm outside the egg gave rise to a considerable disengagement of growth and differentiation processes. Both were slowed, but the former m than the latter. For the formation of one somite under normal conditions *in* between the stages of 2 and 27 somites, 1·04 hr. are required, but *in vitro* time taken was found to be between 2·44 and 1·15 hr., with an average at 1·65 This reduction in the rapidity of somite formation was approximately cons whatever the age of the embryo at the beginning. On the other hand, the gro (measured as increase in embryo length) was very much slowed. “The stag differentiation attained by an embryo”, writes Waddington, “is very lar independent of the absolute size, and if a blastoderm is explanted at an early and thus is affected by the slowing of the growth rate for the whole of its an embryo may be formed which, compared with the normal, is very much small for its stage of differentiation.” Thus, an embryo of 19 somites, wh *in vivo* is 5·3 mm. long, may be *in vitro* only 2 mm. long.

There are many other reports of experimental dwarfism in the literat Mention of one recent one only will be made. Bohn & Drzwina[2] subje the developing eggs of the sea-urchin (*Strongylocentrotus*) to *N*/20 and *N* solutions of phenyldimethylbetaine in sea-water. It was found that if the tr ment was begun at the gastrula stage, there was no stop to differentiation, normal plutei were formed, but less than half the size of the controls. The bet plutei provided a striking instance of a derangement of gearing as betw differentiation and growth.

3·113. Nuclear division without cell-division

The experiments of Hoadley, referred to in the preceding section, were to so extent inspired by the numerous earlier works, dating from about the beginn of this century, in which it was shown that morphological differentiation possible without either cell-division or nuclear division.

Chabry[2], in 1888, first succeeded in suppressing cytoplasmic division with nuclear division, by subjecting ascidian eggs to pressure, and J. Loeb[1] later, us hypertonic sea-water, obtained multinuclear single cells and blastomeres. experiments were carried further by Norman, who found that multiple mit (11–18 nuclei/cell) regularly took place when the eggs of the sea-urchin (*Arba* the fish (*Ctenolabrus*) were made to develop in sea-water containing 2 % gCl$_2$. In the case of *Ctenolabrus* high temperatures (30°) were more effective, d blastodiscs containing many nuclei but without a trace of cell-division could sily be obtained.* Later Morgan[2] criticised some of Norman's conclusions, but r the most part confirmed the possible dislocation of the phenomena of nuclear d cytoplasmic cleavage. Work of his own, moreover (Morgan[3]), on the ect of strychnine on echinoderm eggs, brought to light a similar dislocation, d Driesch[6] found other instances in which abnormal temperatures produced lynuclear undifferentiated masses from fertilised eggs. Barta, too, could courage the production of giant polynuclear cells in explanted rabbit lymph des by subjecting them to conditions of poor oxygenation, and it is said that clear division without cell-division often occurs in explanted malignant cells Varren H. Lewis).

In some forms, especially the arthropods, nuclear division occurs naturally thout cell-division in the ordinary course of development. Thus, in the classical se of *Astacus*, the zygote nucleus divides into some twenty descendant nuclei ich migrate towards the periphery of the egg. The egg then divides into a ies of radially arranged pillars or yolk pyramids each having a nucleus at its ripheral end. The pyramidal blastomeres do not, however, persist, for their ervening planes break down in the central part of the egg, and give a layer of lls surrounding an internal mass of yolk. And a similar state of affairs is found all insects, arachnids and crustaceans (see p. 455).

The most interesting recent work on this subject is that of Sugiyama. Sugiyama[1] scovered that sterile hen's egg-albumen, dissolved in sea-water and aged for a ek at 15°, will completely suppress cytoplasmic cleavage in sea-urchin eggs thout interfering with nuclear cleavage. This will occur whether fertilisation effected naturally or parthenogenetically. The effective substance seems to be pontaneous breakdown product of the protein; it adsorbs readily on kaolin or rcoal, but will not pass through a Chamberland filter; it is destroyed by lerate heating. The effect may be obtained not only with sea-urchins of well-wn species such as *Strongylocentrotus*, but also with such forms as *Diadema*, *peaster* and *Anthocidaris*. Sugiyama then showed that no difference existed the respiratory rate of normal eggs and eggs with nuclear cleavages only J. Brachet's experiments[16] below, p. 512). These interesting facts merit further estigation.

hromatin "growth" and multiplication, without either nuclear or cell-ision (*Polyploidy*) may be added to this section. It may be brought about erimentally by the action of many pure chemical substances, especially chicine (Blakeslee & Avery; Koltzov[5]), acenaphthene (Schmuck; Navashin; stov[1]), bromacenaphthene (Schmuck & Kostov), tribromaniline (Favorski) and er compounds (Schmuck & Gusseva); cf. formulae on pp. 248, 256. According

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ction of low temperatures, about 0° C., also gave the same results, as was shown by the work erassimov on plant cells and Bucciante[1] on explanted heart fibroblasts. Evidently cytoplasmic sion is more susceptible to unfavourable conditions at both ends of the normal range than is lear division.

to the last-named authors, nearly all derivatives of α-naphthalene are active none of β-napthalene. The reader may be referred to an excellent review Kostov[2], and the genetical importance of the phenomenon need hardly emphasised. Its physiological mechanism is under study (Schmuck, Gus & Iljin). The giant or "polytene" chromosomes of *Drosophila* and other ins furnish another instance of chromatin growth, but without even any attem division.

3·114. Cell-division without nuclear division

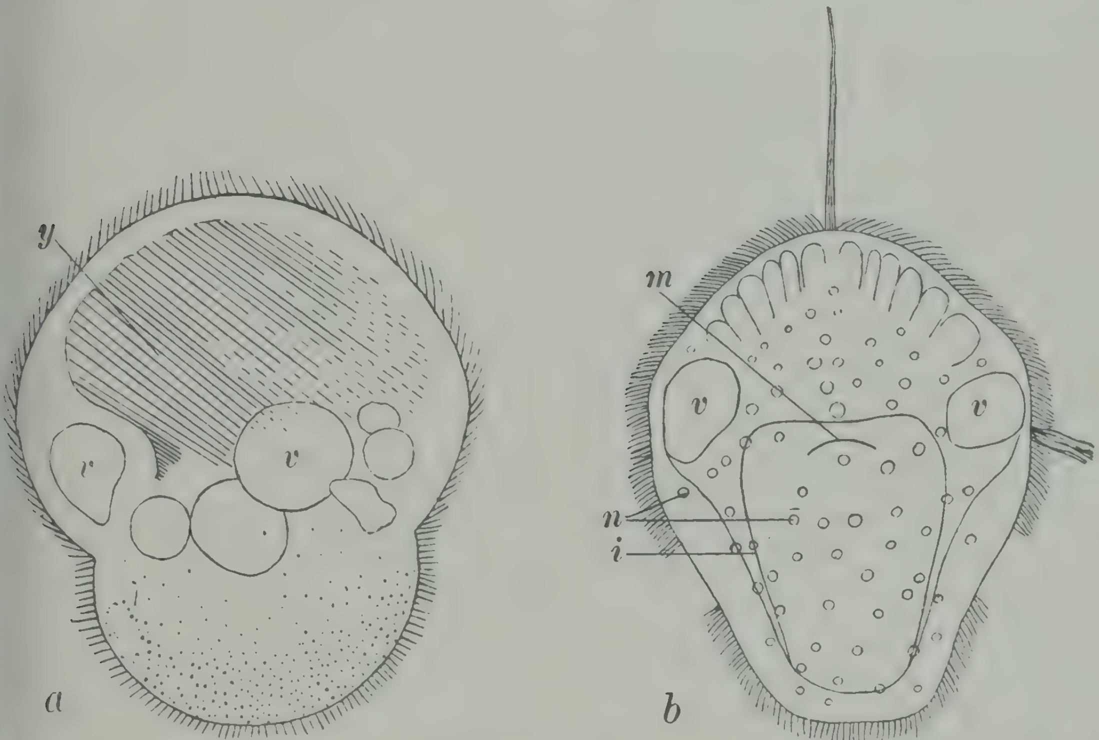
Until a few years ago it would have been thought impossible to write su title. Yet, in the remarkable case of parthenogenetic merogony (discusse Section 2·645), E. B. Harvey has demonstrated the possibility of segmentation even blastula formation in the complete absence of either paternal or mate chromatin, in echinoderm eggs. How far this could be generalised to other fo is a question of much interest.

3·115. Differentiation without cleavage

So far we have only described cases in which differentiation was out of p and cell-division and nuclear division (defined for the present purpose as components of growth) were disengaged. It would seem very unlikely, *a pri* that we could have a differentiation proceeding in the absence of either c division or nuclear division. Yet such a state of affairs was described by F. Lillie[2] in a classical paper in 1902 on the egg of the annelid *Chaetopterus*. A a short exposure to abnormal concentrations of potassium in sea-water, eggs, without dividing into cells, passed through certain well-defined phases differentiation, the yolk accumulating in a dense mass in the interior, and peripheral cytoplasm becoming vacuolated and ciliated. "The ciliated ectopla and the yolk-laden endoplasm", said Lillie, "are analogous to the ectoderm endoderm of the trochophore, and the phases of differentiation resemble so of the normal processes, though the resulting object can by no stretch of term be properly called a trochophore." The phenomenon could be demonstra in both fertilised and unfertilised eggs; and the action of the potassium ion therefore in the first case to suppress certain events which arise normally, in second case to induce certain events which do not normally occur.

The subject is of such interest that two of Lillie's illustrations are reproduced. Fig. 286 *a* shows a well-developed ciliated object from his potass cultures, Fig. 286 *b* a normal trochophore. The ciliated object is radially symmet round its long axis, and is divided by a constriction into a smaller and la hemisphere. The yolk is aggregated in a dense mass in the latter, and a ro large vacuoles completely surrounds the body near the constriction; the pr plasm of the smaller hemisphere is very dense and granular; the cilia are str active, and regularly placed. No cell structure could be detected in the li object. It presents, as Lillie said, "an undeniable resemblance to a trochoph if the smaller hemisphere be compared to the pre-trochal, and the larger to post-trochal region, the large vacuoles occupy approximately the position of prototroch. A similar girdle of vacuoles is found in this position in the troc phore. The aggregation of yolk is in a similar position to the gut of the troc iore.” And on sectioning it was clear that instead of the hundreds of nuclei sible in the normal trochophore, only one large nucleus was contained in such ciliated object.

In a later paper Lillie[3] reported that certain other treatments besides abnormal ’ concentrations would produce the same result, i.e. letting the eggs remain ab- ırmally long in ordinary sea-water before fertilisation (cf. p. 255), or exposing em to abnormal temperatures after fertilisation. Moreover, the unsegmented gs undergoing differentiation might be either mononuclear or polynuclear. nfertilised eggs treated with KCl would be of the former type, fertilised eggs ould be of the latter if they had undergone polyspermy or if they had shown ıriginal cleavage followed by fusion of the blastomeres. It was found that the ·nuclear unsegmented eggs differentiated into the “ciliated object” condition as rapidly as normal eggs produced trochophores, whereas the mononuclear s took a considerably longer time to develop.



286. Differentiation without cleavage in the egg of *Chaetopterus* (rearranged from Lillie) To the left: a “ciliated object” developed from an unfertilised egg (after 24 hr.). Treatment: in 95 parts of sea-water plus 5 parts of 22 N KCl for 1 hr. To the right: a normal trochophore 24 hr. old (same magnification). Reference letters: *i*, intestine: *m*, mouth; *n*, nucleus; *v*, vacuole; *y*, yolk.

haetopterus did not long remain the only animal in which differentiation hout cleavage had been observed, for Treadwell described it in another ielid, *Podarke*, and J. W. Scott found a particularly striking case in yet another, *iphitrite*. Development without cleavage, under Scott's conditions of par- nogenesis, took the form of nuclear divisions, the early differentiation of a r of ectoplasm, the growth of cilia, the appearance of vacuoles that are found the fertilised egg of the same age, the development of brownish pigment, ıeboid movements of cytoplasm, and change in the general shape of the egg.

The apical tuft of cilia characteristic of the normal trochophore was alw absent, however, from these "ciliated objects" of *Amphitrite*, as also from th of *Chaetopterus*. Certain experiments of Bastian on rotifer eggs could not explained, according to Lillie[3], except upon the supposition that differentiati without cleavage was a possibility there also.

This curious phenomenon has recently been the subject of physiologi research by J. Brachet[16], using *Chaetopterus*. The differentiation without cleava was inhibited by KCN and iodoacetate. It was possible to show beyond dou that there is an initial fall of respiratory rate, just as in normal fertilisation (p. 563), but that the gradual rise which follows occurs at only about half t normal rate. The pre-fertilisation level is thus reached by normal embryos $3\frac{1}{2}$ hr. after fertilisation, but by the "ciliated objects" at $6\frac{1}{2}$ hr. Neverthele the fact that any rise in respiratory rate occurs at all is interesting enoug Estimations of thymo-nucleic acid (see p. 633) showed also that the synthe of this by the "ciliated objects" in 8 hr. following fertilisation or activati amounted to only 30 % of that synthesised by normal developing embryos. B the fact remains that there is such a synthesis.

Further work along these lines will be awaited with interest. Although general it is true that no nuclear division occurs in differentiation witho cleavage, there are numerous peculiar nuclear phenomena (description J. Brachet), so that nuclear activity cannot be quite excluded.

A mild case of differentiation without cleavage in the egg of the Zebra fis *Brachydanio rerio*, has been reported by Roosen-Runge. The blastoderm too on its normal shape, but no cell-division occurred within it.

Such considerations lead naturally on to a discussion of the relations betwee cell size and cleavage. Thus Henrici's interesting experiments on bacteria showe that in fresh media some cause operates favourable to increase of cell materi but inhibitory to cell-division, leading the majority of cells to attain an abnorm size before splitting occurs. At the end of the "lag period" of growth, fo instance, the average length of *Bacillus megatherium* was six times that of th cells at the time of inoculation. Gates, too, could abolish cell-division in bacter by ultra-violet light, so that "spaghetti-like" filaments were formed. Simil results have been obtained by G. S. Wilson on other bacteria. But it would tak us too far from the point at issue to deal adequately with the relation of cell siz and cell number to cleavage, and a reference to the recent reviews of H. J. va Cleave and Berrill[5,6] must suffice.

One of the more interesting recent contributions to this question from th embryological point of view is the work of Bhatia on the muscle cells of th developing trout. Bhatia showed that throughout the free-swimming yolk-sa period after hatching, the growth of the muscle cells is entirely due to growth i size, and no cell-divisions take place. But as soon as the larva begins feeding an the yolk has all been used, cell multiplication begins; the increase in cell siz alone cannot now keep pace with body growth. These phenomena call to min the statement of G. P. Wright[1] and others, that avian yolk inhibits mitosis i explanted cells (cf. p. 631).

n the general question of differentiation without cleavage, the conditions in tozoa may be remembered. An extraordinarily high degree of differentiation l morphological complication may exist in unicellular organisms (cf. Calkins).

3·116. Differential action of hormones, amino-acids, etc. on differentiation and growth

The endocrine system affords a powerful instrument for the disengagement of wth and differentiation, as was first realised by Gudernatsch[1] in his classical rk in 1912 on the thyroid control of amphibian metamorphosis (cf. pp. 447 ff.). Vir haben es also hier mit Differenzierung ohne Wachstum zu tun", said dernatsch[2], reviewing his earlier work in 1929. "Wir wissen, dass in der rmalentwicklung des Individuums zwei Processe parallel laufen, Wachsn und Differenzierung. Im Kaulquappenversuch ist es uns zum ersten le gelungen, diese Prozesse voneinander zu isolieren." And indeed the mature metamorphosis induced by thyroxin affords the standard example the disengagement of two fundamental processes (Uhlenluth[3]).*

At the present time attempts are being made to discover a differential action the amino-acids on growth and organisation. It is possible to rear tadpoles to tamorphosis on a diet in which a single amino-acid is the sole source of rogen; carbohydrates, fats and vitamins being given in the form of a basal d. It has thus appeared that the amino-acids differ in their effects on growth l differentiation (Gudernatsch & Hoffman[1]; Hoffman & Gudernatsch). ginine, cystine, lysine and phenylalanine permit growth, but practically no erentiation. Tyrosine and tryptophane, on the contrary, favour differentiation her than growth, and produce small well-developed animals. In certain cases h growth and differentiation may be suppressed, the tadpoles simply conuing without much change, e.g. with leucine and alanine. Certain amino-ds, such as histidine and aspartic acid, hardly permit even maintenance. iodotyrosine brought on the usual early metamorphosis, but this could be ded, it was found, if a typical "growth acid" were given at the same time Hoffman). Gudernatsch & Hoffman[2] are studying the permutations of the no-acids, and Gudernatsch[3] has summarised the whole of the work. Experits of a similar nature have been reported by Pincherle & Gelli.

inally, high oxygen tensions are said to retard the growth of the chick embryo le differentiative processes are accelerated (Demuth[2]).

·117. Disengagement of determination from differentiation and growth

he determinative factor in differentiation can, it seems, be disengaged from growth process just as much as the other factors. In the interesting work of itty[1] (p. 337), who studied the determination of the ciliary beat on the skin of phibian embryos, we have seen how a determinative process can be shifted in experimentally, and thus thrown out of gear with the growth processes.

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it there are many others; the work of Voitkevitch[1] on feather development, for example, at of Gray & Worthing in which the development of the neural system in the chick embryo nibited by tetanus toxin, without the somites being affected.

The well-known observations of Hörstadius[3] on echinoderm eggs also be here. By various operative procedures (alteration of osmotic pressure, etc. was able to shift the relations of cleavage and the determination of regi differences of cleavage type. Cleavage type (i.e. micromere formation) w thus follow the usual course in time in spite of great delays in cleavage, with result that a micromere could be formed with only one vegetal macromere ins of four and only two animal mesomeres instead of eight. These conclusions h been confirmed in parallel work on *Pomatoceros* by Berrill[5].

3·118. Mutual independence of growth, histogenesis and orga genesis

The technique of tissue culture has afforded many new possibilities of stud the relation between growth and differentiation. It was early found by Stra ways in 1924 that successful cultures of metazoan cells *in vitro* proceeded al one of two directions; either cell-division took place side by side with m "outwandering" from the original explant, or cell-division occurred only in explant itself. The first of these processes led to no increase in differentiati and was often called "unorganised growth", the second might involve reaching histogenesis and even organogenesis, and was called "organised growt This "unorganised growth" was formerly thought to involve dedifferentiati but it is now regarded as mere modulation (see pp. 279, 506). "Organi growth" we have already met with under the name of self-differentiat (p. 103). Self-differentiation may include morphogenesis as well as histogene or it may not. The otocyst is a perfect case of the latter, for all the tissues of normal finished product are formed, but no spatial arrangement whatever (Fel Embryonic limb bones show the former type, for a high degree of morpholog or even anatomical differentiation is realised in their explants (Fell[2]). In teratomata we have histogenesis, but organogenesis only in very few.

The independence of histogenetic and organogenetic differentiation has b much emphasised by Ranzi[2,9,10]. In the course of his work on the experimen embryology of cephalopods he often observed that the formation of the differ sorts of cells characteristic of an organ may proceed quite normally, although characteristic morphological form of the organ has been suppressed or modif experimentally. Morphological development was profoundly affected if a *Se* embryo was removed from its yolk and allowed to go on developing, but his logical development proceeded normally.

It is clear, then, that histogenesis can occur without organogenesis; and converse may probably also be true.

3·12. Disengagement of growth, differentiation and metabolism

3·121. Metabolism without growth

Bacterial phenomena do not come strictly within the field of this book, it is hard to exclude a reference to them, since they throw some light on essentials of the present problem. In bacteria differentiation does not come i y, hence the relations between growth and metabolism can be studied without t additional complicating factor. Under natural conditions growth and tabolism are not separated in bacterial life, for if a colony finds sufficient rient materials to permit of existence, it will automatically enter a period of avage. But there are certain cases in which it has been shown that metabolism occur in the absence of growth, so that cell-division is quite absent and the ls are simply maintaining themselves as far as possible in the *status quo.*

The first of these is that of "resting bacteria". The term "resting bacteria" s introduced by Quastel & Whetham in 1924 to mean bacteria which were piring but not growing. The organisms were grown in tryptic broth, separated centrifuging, washed in saline solution, made up to a thick emulsion with ne, and finally well aerated. Under these conditions no proliferation takes ce and the properties of the enzymes present in the cells can be investigated hout the complicating factor introduced by the syntheses of growth. The ults of such investigations have been summarised by Quastel in a review. w far the resting bacteria were alive, however, was questionable from the ginning, and attention was specifically devoted to this point by Cook & phenson. The only available criterion of the life of resting bacteria was bility; would they divide when replaced in nutrient medium? The results of bility counts showed that only from one-third to one-tenth of the resting teria were able to divide when given natural conditions. Oxidations and other ctions produced by the resting bacteria, however, were not due to these viable s alone, for when this viable minority was reduced to 0·02 % of its original ue by exposure to ultra-violet light, the rate of reaction was only slightly cted. Nevertheless, the presence of a considerable proportion of viable anisms among the resting bacteria demonstrated a disengagement of the growth l metabolism processes. It appears that under natural conditions most of the teria in sewage, though chemically very active, are incapable of growth, a state similar to the "resting" bacteria (Wooldridge & Standfast).

A second case is that of crowded yeast cells. Adrian Brown showed in 1892 t with yeast growing in malt wort, the medium would only allow the cells to ease up to a certain limit, and that if this concentration were reached or eded in the initial sowing, no multiplication took place although fermentation ceeded freely. This observation, an earlier example of the "resting" condition, nitted the study of fermentation apart from growth and enabled Brown to nine the effect of oxygen supply, etc., on the fermentation process alone. an account of the subsequent work to which this led, reference may be made ne monograph of M. Stephenson.

third case is that of nitrogen fixation by *Azotobacter*. Iwasaki showed on a medium free from fixed nitrogen, cell-division may occur without ogen fixation and conversely nitrogen fixation without cell-division. The first nese conditions happens when an old culture is diluted with new medium; respiration rises, and cell-multiplication goes on without nitrogen fixation, le the size of the individual cells decreases. After some time, when the popula- has greatly increased, cell-division ceases and nitrogen fixation sets in; allel with this, the cells increase in size. If the culture is very greatly diluted, ultiplication phase occurs as before, but it is followed by a simultaneous tiplication and nitrogen fixation phase, before the colony passes over into pure nitrogen fixation phase. Owing to the dissociation here between cell- sion and increase in size of the cells, the dissociation between growth and characteristic chemical activity is not perfectly clear, but it is obvious th certain measure of disengagement between metabolism and growth is norm this organism. The state of affairs has striking resemblances to that referre below in the discussion of growth and metabolism in explanted cells of hi animals.

The antibody inhibiting cell-division in trypanosomes, but not any of t other normal functions (Taliaferro), might be regarded as a fourth case.

3·122. Metabolism without growth and differentiation

We do not have far to look for phenomena of a similar kind in the embry life of the higher animals. Many insect embryos, as described elsewhere in book (pp. 578 ff.), complete a certain part of their development and t enter upon a state of dormancy or diapause which lasts for a considerable ti and at the end of which a short period of rapid development leads to hatchi The silkworm egg is perhaps the most famous example of this mechanism, it is very common among the orthoptera. But during the diapause the respira of the embryo does not completely disappear; on the contrary, there is a s but continuous maintenance metabolism. The growth and differentiation p cesses have been thrown out of gear with the metabolic processes for the durat of the diapause.

It is not so generally known that something analogous to the diapause occ in mammalian development. According to the work of many observers, review by Hamlett[3], the embryos of many mammals pass a considerable proportion their intra-uterine life in a state of suspended development. There can be li doubt but that some metabolism is proceeding also during this "diapause". reptiles, embryonic hibernation is found in the case of the tuatara lizard, *Spheno* (Dendy), and the pond tortoise, *Emys* (Boulenger). In birds it has not so far be reported, but certain English wild birds present suspicious behaviour from t point of view. It is said that the blackbird, for instance, lays one egg each for a week, but although sitting has begun from the first day, all the eggs ha out together. There are also some subtle adjustments of incubation times parasitic birds such as the cuckoos (H. Friedmann), of which the oecologi value, but not the mechanism, is obvious.

If natural embryonic hibernation is an instance of the possibility of suppress differentiation and growth in a developing system, while retaining a maintena metabolism, so is the viability of metazoan cells at low temperatures. Buccian has examined with care the survival of embryonic cells in these conditio Taking hen's eggs from the incubator at definite times after the beginning incubation, he allowed them to remain at standard lower temperatures a measured the time elapsing before it became no longer possible to make success tissue cultures from the embryonic cells. As the table opposite shows, various tissues have different times of survival.

But more important for the present purpose is the fact that at these low te peratures metabolic processes were proceeding although growth and different tion had been totally done away with. Not much is known about the respirat of tissues at 0°C., but the work of E. C. Bate-Smith[1] conclusively showed that i ot negligible. Amphibian muscle at 0° takes up about 0·025 c.c. of oxygen/gm./hr. vet wt.).

		Time in days before complete loss of viability in tissue culture	
		At 15–20°	at 0°
Skin		24	32
Cornea		18	28
Meninges		21	25
Amnios	epithelium	7	17
	muscle	5	10
Skeletal muscle		21	22
Spleen	fibrocytes	6	12
	leucocytes	3	5
Heart		6	10
Liver	endothelial cells	6	10
	hepatic cells	2	5
Mesonephros		6	12
Mesencephalon		6	10
Aorta		7	10
Iris		6	11

Other workers have carried out experiments similar to those of Bucciante, but eir durations of survival have not usually been as great as his, probably owing the fact that they have placed the embryos or parts of embryos in saline solutions the low temperatures instead of keeping within the intact egg. Thus, Verne & diette used Tyrode solution and found the survival was improved if glucose ere added to it. Bucciante[5] himself considers that the saline solution washes t from the tissue certain necessary growth factors. A similar difference technique explains the long survivals of whole rat embryos reported by monin as opposed to the short survivals of S. Kodama. Nicholas[1], again, has ccessfully kept whole rat embryos at 24° in Ringer solution for 72 hr. with no gn of differentiation or growth, but no doubt considerable respiration.*

One point which deserves mention here is that the development of tissues may unequally affected by exposure to low temperature. Coghill[2] states that in phibia ectoderm is much more sensitive than mesoderm.

3·123. Stepwise inhibition of growth, differentiation, fermentation and respiration

So far, abnormal temperatures and naturally adjusted processes alone have ered into the description of the disengagements which may occur between owth and differentiation on the one hand and metabolism on the other. But re are other agents which may be used experimentally to affect the gearing.

Of these the classical example is the work of Warburg[1]. In 1908 he studied the ect of hindering or inhibiting the cleavage of sea-urchin eggs by placing them hypertonic sea-water. Although profound effects were observed on the segentation, the respiratory rate was very little changed (0·368 mgm. O_2 hr. mgm. egg nitrogen in the normal lot, 0·347 in the inhibited lot). In a later per he described the effect of phenylurethane. Under the influence of this rcotic, segmentation could be wholly abolished, and yet the respiratory rate

* Cf. p. 450.

remain unaltered, if the dose was well chosen. Thus, after 25 min. in ordin
sea-water the astrospheres are visible, but nothing has happened in N/2
phenylurethane. At 40 min. the first cleavage takes place in normal eggs, but
the phenylurethane eggs the astrospheres are only just appearing. At 90 m
the second cleavage takes place in normal eggs, but in the phenylurethane e
only the equatorial plate stage has been reached. Yet in spite of this great retar
tion of cell-division, the respiratory rate was in no case affected to a higher deg
than 20 % (e.g. 0·450 c.c. O_2/hr./28 mgm. egg nitrogen in the normal
0·438 c.c. in the phenylurethane lot). "The visible changes in the early develop
egg", as Warburg said, "are not conditions of the oxygen utilisation follow
fertilisation. But the oxygenation is a condition of the visible changes, so t
those chemical processes, the activity of which we can judge by the amount
oxygen consumed, would seem to underlie the morphological ones."

In this connection it is striking that the cleavage of *Arbacia* eggs is complet
suppressed at a CO_2 tension of 120 mm. Hg (Haywood & Root[1]), but th
oxygen-consumption is still 30 % of the normal value and remains so up
180 mm. Hg (Root).

Experiments which show an identical disengagement of the fundamen
processes were made in 1930 by Partachnikov. Partachnikov studied the act
of quinine on heart fibroblasts of the chick growing *in vitro*, and found that t
inhibitory effects on growth and on metabolism (as assessed by the disappearan
of glucose from the culture medium) ran by no means parallel.

	Growth (Ebeling index)	Glucose utilisation %
Control	3·96	71
Quinine: 1 in 10,000	0·33	58
1 in 5,000	0·0	34
1 in 2,500	0·0	0

Thus, at a certain concentration of quinine the growth (cell-division, increase
cell size, etc.) was inhibited 100 %, the metabolism only 55 %. Here grow
was more vulnerable than carbohydrate breakdown.

A similar stepwise inhibition is seen in experiments with iodoacetat
(Krontovsky[2]; Krontovsky, Jazimirska-Krontovska & Savitzka) and with radiu
emanation (Mikhailovsky). Mikhailovsky cultured explants of embryo heart
much the same way as Partachnikov, but subjected them to the action of radiu
emanation for periods ranging from 4 to 20 hours. Again the inhibitions of grow
and metabolism were not parallel.

	Area of explant sq. mm.	Glucose utilisation %
Control	14·15	79
4 hr. irradiation	7·5	67
20 hr. irradiation	—	53

So in this case the inhibition of growth was 47 % and that of glucose utilisati
15 %.

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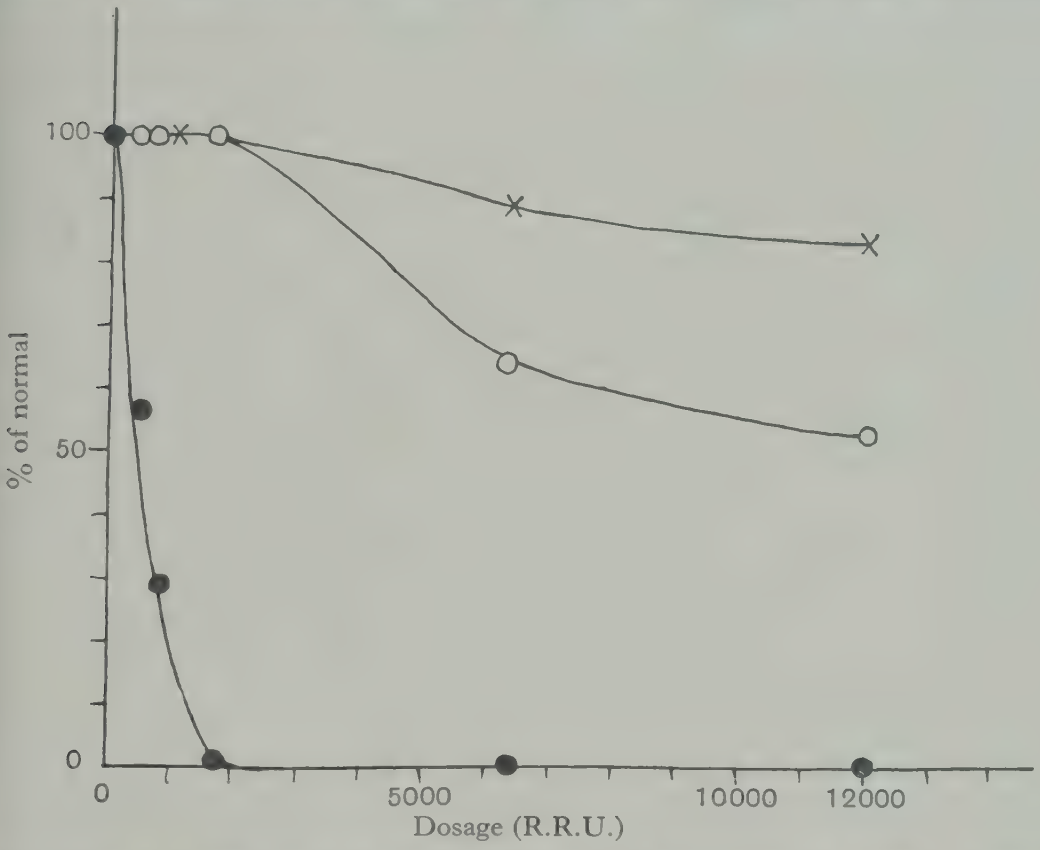
* It may be of interest that Soliterman has detected changes in vital staining properties of
cells which coincide with bromacetate inhibition of glycolysis and not with cessation of grow

Hubert's work on the chick embryo was particularly interesting, in that he easured the inhibitions not only of growth and respiration, but also glycolysis, used by irradiation with X-rays. He subjected 5th-day chick embryos while ll *in ovo* to the action of accurately dosed X-radiation, and then measured colysis and respiration by Warburg manometer methods on the same day, and owth by leaving a population of embryos until the 12th day and then observing eir fate. Fig. 287 shows the results. It is clear that the effect of X-radiation on the fundamental processes was not at all the same; growth was the first to affected, then glycolysis, and finally respiration. The strongest treatment of



287. Effect of X-rays on chick embryo (Hubert). ○ glycolysis, ● growth, × respiration.

(12,000 Röntgen radiation units) caused only a 15 % decrease of the piration below normal, but glycolysis was reduced by 45 % and growth irely stopped.

-radiation is therefore an agent which at a certain level will abolish growth, cause no change in the intensity of respiration or fermentation, while at ther level it will exercise an inhibiting influence on these metabolic processes well. The parallel with Warburg's early experiments is striking.

imilar data exist for tumour cells. Crabtree[1] irradiated Jensen rat sarcoma h γ-rays. At 10 hr., respiration, but not aerobic glycolysis, was depressed. 18 hr. both were depressed. It seems also that doses lethal for tissue cultures normal mammalian cells do not depress their glycolysis (Krontovsky[1]; Kron- sky & Lebensohn). And this can be seen in *Arbacia* or *Chaetopterus* embryos, ere L. Chesley found that developmental anomalies occur at doses of γ-radiation te insufficient to inhibit normal or dye-stimulated respiration.

So far the examples of this section have all been concerned with develo embryonic cells and tumour cells, but there are examples from unicell organisms which fit in closely with the argument. Firstly, the effect of X- on the fermentation, respiration and division of yeast cells was investigated Wels & Osann. Here again a marked differential effect was obtained, gro being by far the most susceptible of the fundamental processes, and the o two, indeed, being hardly affected by the doses used. Fig. 288 summarises data. Just as with the chick embryo in Hubert's experiments, growth (divisi is abolished before any effect has become evident upon respiration or fermen tion. It was interesting that cell-free fermentation showed the same immun to X-ray damage as fermentation by the intact cells.

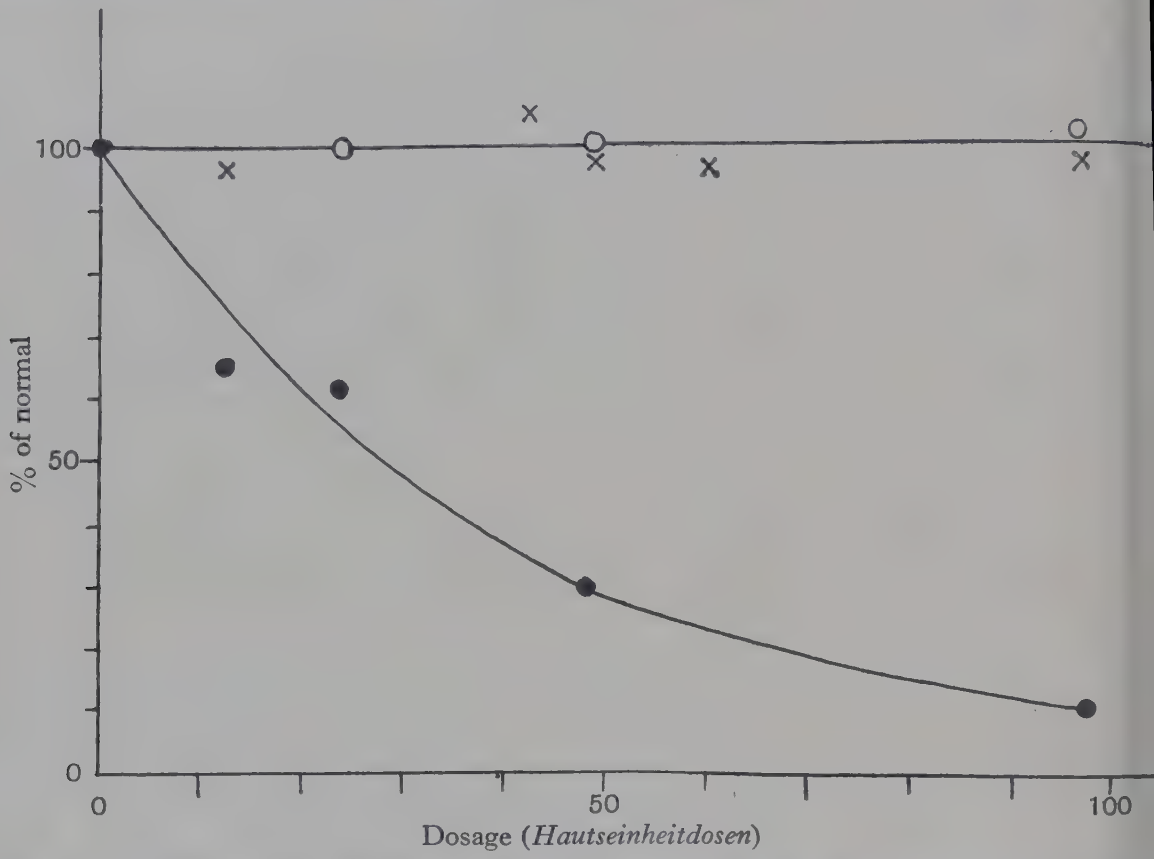


Fig. 288. Effect of X-rays on yeast (Wels & Osann). ○ respiration, × fermentation, ● gro

Similar experiments to those of Wels & Osann were carried out by Rub as part of a general survey of the role of water in the living cell. By placing ye cells in different concentrations of salt, he reduced their water-content and—wh the concentration was sufficiently raised—their heat production. But onl mild degree of hypertony was required to stop growth, and as the data in Fig. show, in 4 % salt solution growth is almost completely suppressed while wat content and heat production continue at almost their normal level.

By differential exposure to radiation a veritable "dissection" of metabolism possible. Using γ-radiation on embryonic rat kidney growing *in vitro*, B. Holmes[1] found that protein catabolism (as judged by ammonia production) much less sensitive than carbohydrate catabolism (as judged by lactic a duction), the former continuing unaffected when the latter was reduced to % of its normal value. She next showed (B. E. Holmes[2]) that in the same tissue, inase, an enzyme significant in view of the ornithine cycle (p. 52), was affected by a dose of γ-rays capable of reducing the activity of lactic dehydro-nase over 50 %. Moreover, glucolysis (with glucose as substrate) is much re severely affected than glycolysis (from hexosediphosphate) in tumour tissue E. Holmes[4]).

arallel experiments were made by Crabtree[2]. Using mixed β- and γ-radiation tumour tissue, he discovered a temperature effect. At 37° respiration was much re vulnerable than anaerobic glycolysis; at 0° the reverse was the case. But oxalase, like arginase, was unaffected at any temperature. Crabtree[3] extended

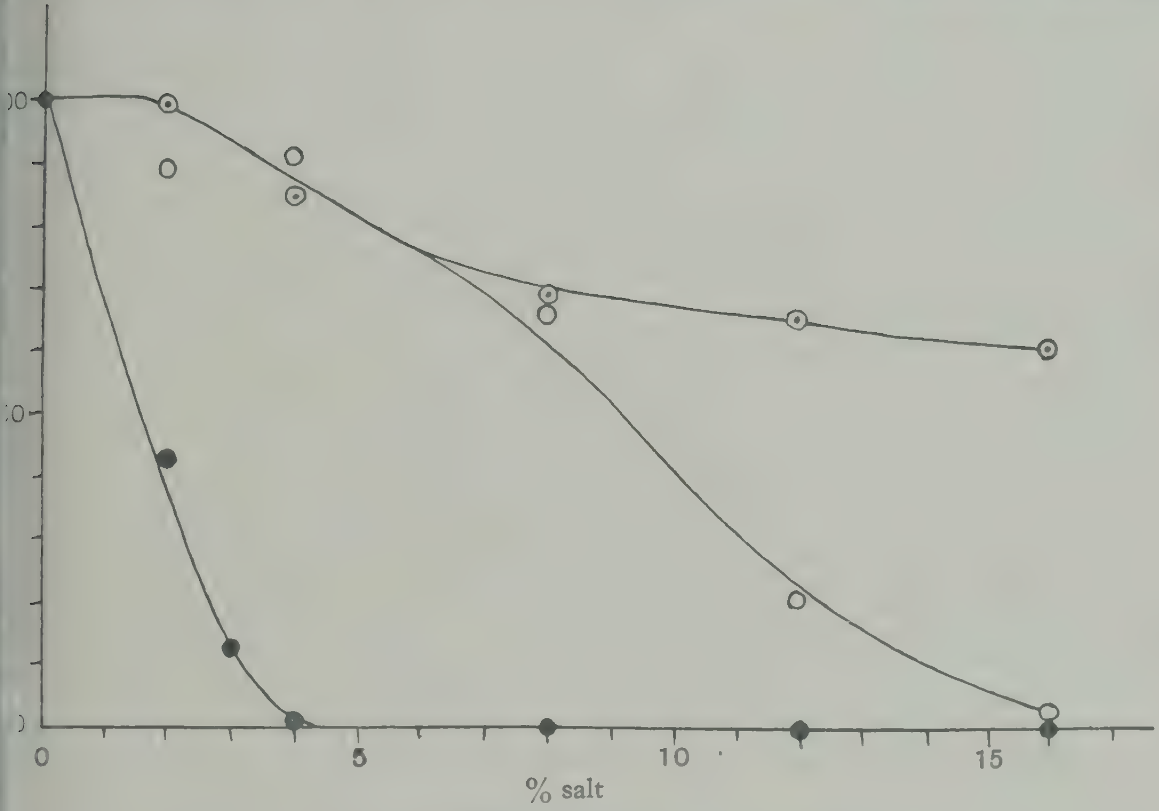


Fig. 289. Effect of hypertonic solutions on yeast (Rubner).
⊙ water content, ○ heat production, ● growth.

former results on protein catabolism by showing that ammonia production umour and normal adult tissue is actually stimulated 100 % by β- and γ-ation, independent of what has happened to the carbohydrate catabolism, whether glycolysis has been reduced to nil by irradiation at 0° or whether it is fully active after irradiation at 37°. Crabtree, however, views the stimulated tein breakdown as compensatory for the lost power of carbohydrate break-vn (cf. work on grasshopper (p. 581) and chick (p. 592) embryos). Stimulation rotein breakdown in this way has also been found in the case of the protozoon *o caudatus* by Lawrie & Robertson. Crabtree & Cramer discovered the resting fact that the radiosensitivity (diminution of lethal dose) of tumour ie is increased if respiration is inhibited by anaerobiosis, KCN, or low temperature, but not if glycolysis is inhibited by iodoacetate or fluoride. T fits in with Lipmann's finding[1] that the growth of embryonic heart fibroblast inhibited by partial anaerobiosis 50 % when the Q_{O_2} is reduced only 30 %, suggests again that respiration is more important than glycolysis for grow Conversely, Kolomjetz found with radium emanation on the same material t growth was reduced only 34 % when glycolysis (as judged by lactic acid prod tion) was decreased 50 %.

3·124. Cell-division and differentiation without oxidative metaboli

Cases of the disengagement of growth or differentiation from metabolism h so far been restricted to the removal of the former while the latter remains int But is it possible for growth and differentiation to proceed in the absence metabolism? In the profoundest and most inclusive sense in which the w metabolism can be used, it is probably not possible, but there are not want suggestions that the early stages of development may be facultatively anaero If this were so, the whole series of mechanisms within the cell which carry out oxidations would be dissociable from the processes of growth and differentiati which would compulsorily engage only with fermentative reactions or w reactions involving hydrogen acceptors other than oxygen.

Thus, as we shall see (p. 564), many if not all the reactions of fertilisation possible anaerobically in some marine eggs. On the other hand, experime suggesting that the echinoderm egg can develop using dyes such as methyle blue instead of oxygen have not been confirmed. The special case of nemat eggs may be relevant here. Although the cleavage stages are in general aero (see p. 576), Dyrdovska now claims that the first cleavage can occur in p nitrogen.

As regards the chick embryo, little is known, in view of the experimental di culties attending the study of it in the earliest stages. But M. T. Burrows discove in 1920 a remarkable change in the respiratory properties of the chick emb with age. Taking explants of chick embryonic heart cells, he placed them different partial pressures of oxygen and found that their behaviour was qu different according to the stage of development of the chick from which they h been taken. Fibroblasts from 4–5-day embryos would grow and pulsate 46 hr. or so in pure nitrogen, and for 50 hr. in only 7·5 % of oxygen. Fibrobla from 10–15-day embryos, however, would not grow at all in nitrogen, and o for 12 hr. in 1·5 % oxygen. This must mean that the younger cells possess so source of energy which the older ones do not have. Burrows' work was la confirmed and extended by Wind, who paid particular attention to strictness anaerobiosis. In his conditions only the smallest amount of growth proceed even with 4–5-day embryos, but at an oxygen concentration of 2×10^{-4} vol. % difference was clear-cut, for cells from 10-day embryos would not grow at while those from 5-day embryos grew profusely. Wind also made the interesti observation that if the cells were in each case subcultured for several weeks a then brought under anaerobic conditions, the difference between 5-day and 10-d embryos had disappeared.

There is certainly no truth in the statements of the earlier workers that avian ›velopment may proceed to any length anaerobically. But *unorganised* growth such circumstances undoubtedly occurs (Byerly[1]).

3·125. Selective engagement of growth or differentiation and characteristic chemical activity

In the preceding paragraphs we have been considering the disengagement of owth and metabolism only in the sense of maintenance metabolism. But there e many other chemical reactions performed by the cells of an embryo, such as e storage of glycogen or the elaboration of a characteristic pigment, and it is in der to enquire what relations exist between these special types of metabolic ange and the growth processes.

On purely morphological grounds, Peter[1] and Lauche were led to the conclusion at "a cell which is working does not divide, and a cell in mitosis is not working".* ne subject entered a new phase when Fischer & Parker described a technique r regulating the intensity of proliferation of tissue cultures, for it was found at osteoblasts, growing under the conditions of "vie ralentie", formed bone, hough, as Doljanski[1] had previously shown, they would not do so when ltivated in the normal way. This indicated that growth and characteristic emical activity might be mutually incompatible in tissue cultures, as if a imary shaft could engage with one of two adjacent gears, but not with both.

These considerations led Doljanski to make a closer study of the relations tween growth of explanted iris, and melanin production. Explanted iris, if pidly growing and repeatedly subcultured at short intervals, had been found Kapel not to produce pigment. Doljanski[2] now took pure cultures of iris ithelium from the chick embryo and cultivated some in the usual embryo tract, others in adult fowl plasma. The latter grew much more slowly, of course, an the former. But "while the intensely proliferating culture in embryo extract tained at the end of some time few or no pigment granules, the culture whose wth had been inhibited showed compact masses of black pigment. The nules of melanin accumulated in great quantities in the central part of the ›ny, and were found dispersed throughout the whole cellular membrane." janski deduced an "antagonisme entre la multiplication et la fonction physioque de la cellule".

recisely the same state of affairs was found when liver explants were examined ›ljanski[3]). Ordinarily, when proliferation is rapid, the glycogen contained in original explant soon disappears and no more is formed. But when cultures e transferred to adult fowl plasma, so that growth practically ceased or was y much slowed, glycogen was visible in abundance when the cells were treated h iodine vapour after 12–14 days. Thus hepatic cells in pure culture certainly ıserve their glycogenic power, and it only appears to be absent because wth *in vitro* is usually too rapid to permit of the characteristic chemical ction of the cells. Support for this view could be found in the work of various

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hus, for example, cells in mitosis are not found in developing kidney tubules if these are tional. And Chlopin found that human embryo epidermis would *either* grow *or* differentiate.

histochemical investigators such as Jordan, who concludes that the amoun glycogen in a tissue is inversely proportional to the intensity of cell-division i

Another instance of the disengagement of development and characteri chemical activity might be found in the work of Pozerski & Krongold, as e as 1914, on intestinal grafts. Krongold first showed that the embryonic intest of the rat, grafted to a position under the skin of the adult animal, gives ris a completely differentiated system, all the normally appearing kinds of c (fusiform cells, muscle fibres, glands of Lieberkühn, mucous cells, Brün glands, etc.) being arranged around a central lumen, the whole forming a k of vesicle. But in spite of this display of histological self-differentiation, characteristic chemical products, saccharase, maltase, lactase, and secretin, w found by Pozerski & Krongold to be entirely missing. Enterokinase alone co be demonstrated, and even this, according to Pozerski & Krongold, was m probably of host origin, and was merely being excreted into the vesicle becaus its chance presence in the host's blood stream. Histological differentiation, then, in this case unaccompanied by the appearance of characteristic chemical acti

On the general question of this subsection, Fischer[2] has assembled furt relevant facts in a review. What was proved by Doljanski for explants of iris a liver finds its parallel, it seems, in explants of nerve, heart, and connective tiss according to the results of other observers. Berrill[5], too, has amassed evide from his extensive data on ascidian development that cell-division (growth) a differentiation occur in separate phases.

3·126. Disengagement of growth or differentiation from chara teristic chemical composition

The question now arises whether every morphological stage of developm is characterised by an absolutely definite chemical composition. In general may be said that the limits of "play" here are small. As will later be seen in section on relative chemical growth, or heterauxesis (p. 559), there are as relatively few data on the chemical composition of organisms which have be made to grow fast or slow, and which therefore are of the same total weight differ in age. But they indicate that following a chemical ground-plan of grow the composition follows size or weight, not age. On the other hand, there i remarkably clear case, which we owe to Briggs[1], where in amphibian embr developing at different temperatures the rate of density decrease (due to upt of water by a non-cleidoic egg, cf. p. 33) follows a different course from rate of morphogenesis. At both high (29°) and low (10°) temperatures decrease in density between fertilisation and a given stage of gastrulation is grea than at intermediate temperatures, so that water-content of the system must v independently of morphogenesis. At the same time it should be remember that a holoblastic embryo constitutes a mixed system embryo+yolk, and hen it may not be fair to compare it with embryonic tissues proper where the chemi composition is probably more rigidly fixed. Indeed, the data of Gallow analysed by Briggs, show that in larval life the water-content of the tissues independent of temperature and would vary only with growth.

3·13. Dedifferentiation and degrowth

The analogy of gearing cannot be followed far without involving the concept f reversibility. And indeed it appears from a survey of the known facts that considerable degree of reversibility does exist in the cases of growth and ifferentiation. The question of greatest interest for the present discussion is hether the phenomenon of disengagement is shown when the processes are nder reversal, just as it is when they are proceeding normally from homogeneity heterogeneity and from smallness to largeness.

When an animal organism regresses after reaching a certain stage of develop-ient, its regression may, we find, take one of two forms; either it may consist nly of a reduction in size, or it may also undergo marked morphological changes. he former of these alternatives could be called pure degrowth, the latter egrowth plus dedifferentiation. Of the first type several examples are known, ut no doubt the most famous is that of the starved planarian worm, which was horoughly worked out by F. R. Lillie[1] in 1900. When *Planaria dorotocephala* is arved, it becomes smaller and smaller until it shrinks to a size less than that which it originally hatched from its egg. But the only morphological changes hich occur are slight alterations of the proportions of the parts, and with regard these Abeloos has shown that they retrace the alterations of the proportions parts which accompanied normal growth. Thus measurements of the ratio e-ocular length/total length fall upon the same curve whether the worm is owing or degrowing, and according to Abeloos & Lecamp the same statement plies to the relative size of the epithelial cells of the gut. This reappearance of e characteristic heterauxesis during degrowth enhances our opinion of the versibility of the process, but there are senses in which it is not truly reversible. beloos has demonstrated that the regressed planarian has not become revenescent as regards growth rate; the high growth rate of normal early life is ver regained, and subsequent size increase, brought about by the cessation of rvation conditions, proceeds only slowly.

There is thus no true dedifferentiation in the regression of planarians—unless term were used to refer to the disappearance of the terminal branches of erticulated organs, which occurs in the later stages of reduction. On the ntrary, such special differentiations as the existence of subsidiary eyes persist ring reduction even when the worm has sunk below the size at which it hatched. llie was inclined to think that reduction of cell number was alone concerned regression, but Abeloos & Lecamp show that cell size is reduced too.

More common, perhaps, is the association of dedifferentiation with degrowth. e phenomenon has been known among ascidians for a considerable time; thus, gression in colonial ascidians was discussed by Caullery[1] in 1895 and by Driesch[11] 1902. For the present line of argument the work of Huxley[6] on regression in e ascidian *Clavellina* is of particular interest. His illustrations of the stages in regression are reproduced here in Fig. 290. Fig. 290*a* shows "a perfect little *avellina*, highly contractile, with active gill cilia and wide-open syphons, the ues all as clear as crystal. The first sign of reduction is the permanent con-

traction, followed by the total closure, of the syphons, accompanied by a gen
shrinkage (Fig. 290*b*). After a time the gill cilia stop working and the gills pre
somewhat the appearance of buttons. Then follows a marked shrinkage, acc
panied by loss of transparency and usually by the appearance of numbers of
characteristic white pigment cells (Fig. 290*c*). The opacity obliterates the gill
in the living animal, and the shrinkage leads to the pharynx and peribranc
cavities pulling away from the ectoderm at the syphons; the one process ta
place first in some cases, the other in others. More shrinkage, with consequen

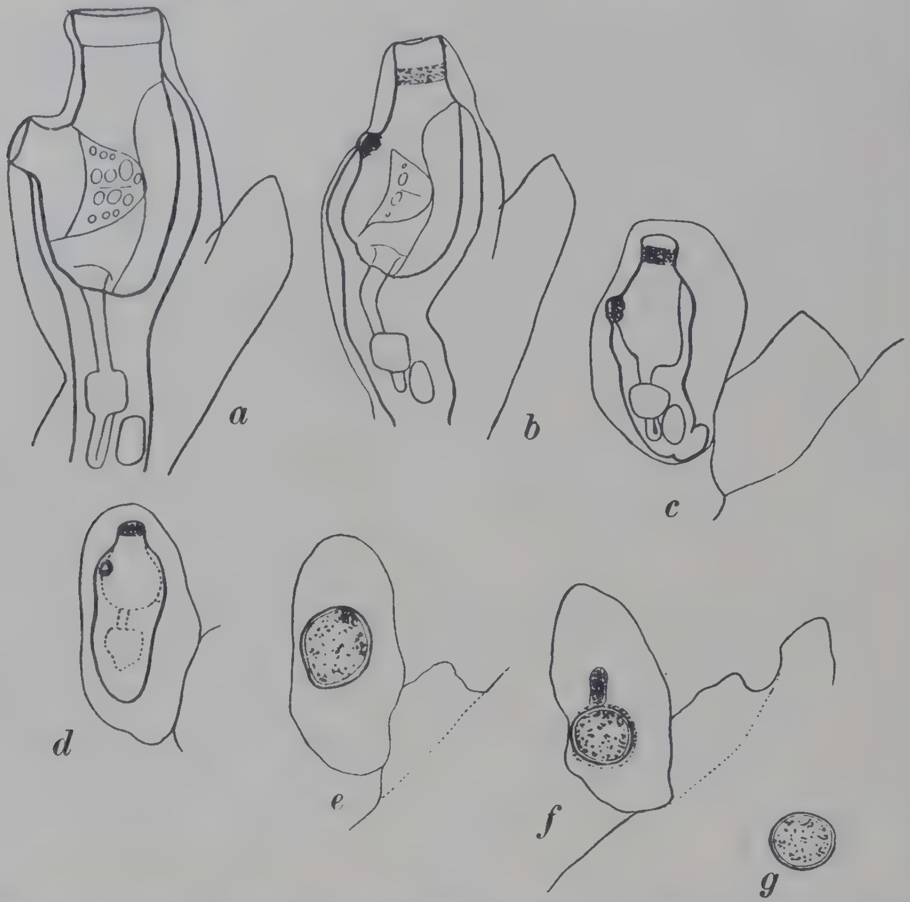

Fig. 290. Regression of the ascidian, *Clavellina* (rearranged from Huxley). (*a*) After 5 da
practically normal and still actively functional. (*b*) After 7 days; exhalant syphon closed, inhal
syphon contracting, gill slits reduced. (*c*) After 9 days; both syphons closed and withdra
within tunic, gill slits no longer visible. (*d*) After 28 days; heart no longer beating, internal org
no longer clearly visible. (*e*) After 37 days; a pigmented ovoid much reduced in size. (*f*) A
46 days; further shrinkage. (*g*) After 53 days; tunic omitted, slight further shrinkage.

greater opacity, brings us to a somewhat dumpy, sausage-shaped conditi
(Fig. 290*d*). The heart, after beating ever more slowly and more irregularly in t
dense mass of loose cells which fill the body-cavity, now ceases its acti
altogether." The organism will now have a small tail if it began with a fai
long stem stolon, but Fig. 290*e* shows a tailless example. "In the next stage, t
tail, if present and not too large, is absorbed entirely, and the long oval or sligh
dumbbell-shaped body of the creature shrinks into a very dense white b
spherical, ellipsoidal, or egg-shaped. Reflected light shows it to be of a yellow
cream colour with milk-white pigment cells dotted over it; by transmitted li
the pigment cells show black on a greenish ground, and here and there in t

ise mass vague clearer spaces are seen, showing that organs and cavities still sist within (Fig. 290*f* and *g*)." Such is the process of degrowth and dedifferen- ion as described by Huxley, and it should be noted that it is not taking place some organism low in the taxonomic scale, but in no less an animal than one the Protochordata. Starvation and toxic agents of all kinds will produce ression, but in this particular case mere confinement in an unchanged volume sea-water was sufficient to produce it.

Now the illustrations of Fig. 290 were all drawn to the same magnification, and s therefore interesting to note that there is a rather sharp distinction between ges *d* and *e*. At first it would seem that degrowth occurs without dedifferentia- n, for stage *d* differs from stage *a* mainly as regards its size, but later on lifferentiation sets in, and the disappearance of organs and morphological m ensues. The examination of regression from the point of view of a com- ison between dedifferentiation rate and degrowth rate would be highly irable. The data required for any quantitative analysis of this relationship do seem to exist as yet in the literature.

n Huxley's opinion, the regressive changes observed in *Clavellina* cannot be posed to represent reversions to stages passed through in embryogenesis. No idian tadpole makes its appearance when an ascidian dedifferentiates. The ef cell change which occurs during dedifferentiation is a regression to the oidal or spherical state, since such a condition requires the least amount of rgy for its maintenance. These conclusions were also reached by Huxley[1] in work on regression in the colonial ascidian *Perophora*. But regression must be in he sense a true reversibility, since it is a passage from complexity to simplicity, n heterogeneity to homogeneity, from high to low organisation, from largeness mallness, and therefore the exact opposite of embryonic development.*

Iany other studies of dedifferentiation throw light on the points discussed ve (e.g. for echinoderm plutei, Runnström; Huxley[2]; for Hydrozoa, Huxley & Beer[1]; de Beer & Huxley; for Amphibia, Blacher). Some investigators are ned to regard the regressed conditions as more closely allied to embryonic es than others will admit. Thus, Hadzi in 1910, subjecting the medusa *saora* to starvation, obtained "gastraea-like bodies" only 80 μ in diameter, posed of cells quite embryonic in appearance. The most remarkable case, ever, is probably that of Davydov, who studied regression in the nemertine n, *Lineus*. By keeping portions of the worm in darkness in a constant volume a-water under starvation conditions for long periods (up to two years) a stage reached at which the size and shape of all the organs made the animal hardly nguishable from an embryo in the pilidium. There was no anus, the brain enormous, and the lateral nerve cords ran immediately under the ectoderm. he final stage (at which Davydov had only two specimens left) a "blastula" ump of large cells was obtained.

t is clear, then, that degrowth can exist without dedifferentiation, and that the s of degrowth and dedifferentiation in a given case of regression by no means

id. moreover, the regressed ascidian will retrace its steps to normality given favourable itions.

necessarily run parallel. Is dedifferentiation without degrowth possible? It wo
mean the collapse of cellular organisation and morphological form without
decrease of mass; the biological analogue of what would occur, as Robert Bo
thought, if the divine sustaining power were withdrawn from the unive
“reducing it to a sort of Chaos or confused State of shuffled and depra
Things” (1744). It might involve both histological and morphological collap
or it might involve morphological collapse only. Both cases occur in nature,
former in the metamorphosis of holometabolic insects (discussed in detail
pp. 470 ff.), the latter in the dissociation and reunition of sponges.

Dissociation and reunition in the sponges has been known for a very long tin
but the facts are as extraordinary as those of insect metamorphosis. If a spon
is dissociated by being pressed through a sieve of gauze, the individual cells
sown on to a glass or other surface, will come together again and in time will fo
a new sponge fully provided with canals, chambers, surface epithelium, skelet
and all the different varieties of cells. Recent papers of Wilson & Penney a
Wilson[2] give a clear account of the process. The normal sponge consists of t
following histological elements: (*a*) epithelioid syncytial sheets lining the exteri
and canal surfaces, (*b*) nucleolate mesenchyme cells, (*c*) “grey” mesenchym
cells, (*d*) rhabdiferous mesenchyme cells, (*e*) globiferous mesenchyme ce
(*f*) fibre cells, (*g*) reproductive cells, (*h*) scleroblasts and spongoblasts connect
with the skeleton, (*i*) flagellated collar cells. The pressed-out material is, of cour
primarily characterised by the fact that the previous morphological organisati
has gone and the cells are isolated, or at any rate only bound together in ve
small groups. But the number of cell types in it is also limited; it contains o
(*b*), (*c*) and (*i*) of the above list, though occasionally (*e*) or (*g*) may be present
negligible proportions. In all cases (*a*), (*d*), (*f*) and (*h*) stay behind with t
skeleton on the gauze. Yet in spite of this absence of constituents norma
essential to the life of the sponge, the reunition mass succeeds in time in produci
new examples of the missing classes, presumably by the specialisation of certa
of its elements. Such elements (probably the mesenchyme cells) must posse
throughout normal life an undiminished embryonic potency or competen
similar to that which attaches, it is suggested, to those mesodermal ce
of the amphibia or other higher animals responsible for regenerations of l
members.

The dedifferentiation of the dissociated sponge, then, consists, first, in t
fact that the morphological arrangement is totally destroyed by the passa
through the sieve, and must be made anew, and, secondly, in the fact that t
population of cells passing through is not representative of the original spon
We cannot quite be said, therefore, to be dealing with pure morphologi
dedifferentiation, but the histological dedifferentiation is statistical, not individu
and thus of a very different kind from that of the histolysing cells of the ins
pupa. Regeneration after dissociation does not consist primarily in the sorti
out of already differentiated cells which take up their former positions as in t
normal organism. The collar cells certainly behave in this way, but the mese
chyme cells (though some of them will remain unaltered) metamorphose into t
various types of cells whose former representatives have remained behind.

The above account of this fascinating subject should be supplemented
reference to the recent papers of Fauré-Fremiet[2]; Brøndsted[1]; Brien[2]; Brien
Meewis; Meewis.

It was formerly usual to regard the growth of cells in tissue culture as involving differentiation, but many workers (e.g. Fischer[1], Törö[2]) have shown that this only apparently so. "Cultivated gland cells of the intestine", writes Weiss ,p. 468), "and pigment cells of the eye, may become very similar in shape and neral conduct, but under proper conditions the former will resume the oduction of digestive enzymes and the latter that of black pigment." Such ersible changes are called *Modulations* (cf. pp. 279, 506, 514).

3·14. Integration or engagement of the fundamental processes

The use of analogy, dangerous though it undoubtedly proved in mediaeval nds, is in our time perhaps almost underrated. An analogy, says Dingle, erely, is at best worthless and at worst misleading. The analogue, not being ntical with the point to be illustrated, has certain relevant and certain irrelevant ments. If the reader does not already understand the point in question, he s no means of distinguishing between them, and if he does understand it, the alogue is superfluous. However, as even Dingle admits, there is something to said for analogies. Previous experiences spring to the mind when an analogy put forward, and the art of choosing an analogy consists in making sure that se psychological overtones will as far as possible correspond with the relevant ments of the analogue. And, above all, in certain circumstances an analogy y provide a framework or co-ordinate net, in which a previously inchoate ss of information may advantageously be assembled.

In the course of this section I have attempted to use the analogy of mechanical rs to co-ordinate a considerable number of facts concerning the fundamental ocesses of ontogenesis. It has been pointed out that:

1) Growth can occur without differentiation (explanted embryonic cells; dian sauropsid embryos).

2) Differentiation can occur without growth (chorio-allantoic grafts; avian elopment *in vitro*; tissue cultures; various forms of experimental dwarfism).

) Nuclear division can occur without cell-division (normally in arthropod ; experimentally in echinoderm and fish eggs).

) Cell-division can occur without nuclear division (parthenogenetic mero- y in echinoderm eggs).

) Differentiation can occur without nuclear and cell division (normally in ozoa; experimentally in annelid and rotifer eggs).

) Endocrine factors can exert a differential action on differentiation and wth (amphibian metamorphosis).

) Dietary factors can exert a differential action on differentiation and wth (amphibian metamorphosis).

) The time of engagement of determinative processes with the processes of wth and differentiation can be altered (ciliary polarity in amphibian develop- nt; determination and cleavage in echinoderms).

9) The relation between growth and differentiation can be affected by genetic specific factors (large and small races of rabbits and fowls; large and small cies of birds; see pp. 420 ff.).

o) Histogenetic differentiation can occur without organogenetic differentia- (tissue cultures of mammalian and avian cells; yolkless development of alopod embryos).

(11) Metabolism can occur without growth (crowded yeast cells; rest bacteria; nitrogen-fixing organisms).

(12) Metabolism can occur without growth and differentiation (insect embr in diapause; reptile and mammal embryos in hibernation; cells of mammal and avian embryos surviving at low temperatures).

(13) Cell-division, growth, respiration, fermentation, and heat product show stepwise inhibition under the influence of chemical agents and radi energy; growth and differentiation disappearing first, then fermentation, th respiration and heat production (yeast cells; echinoderm embryos; bird embry

(14) Growth and characteristic chemical activity may be mutually inco patible, one or the other being engageable with the basal metabolism at one ti (explants of avian and mammalian tissues; histological analysis of tissues).

(15) Cell-division may occur without oxidative metabolism (nematode e bryos; asphyxiated chick embryos).

(16) Growth, differentiation, and metabolism manifest a certain independe of rhythm even in the intact organism (temperature characteristics of growth a respiration, *CE*, p. 524).

(17) Degrowth can occur without dedifferentiation (planarian worms).

(18) Degrowth and dedifferentiation may not run parallel in regressi (colonial ascidians).

(19) Morphological dedifferentiation can occur without degrowth (dissoc tion and reunition of sponges).

(20) Morphological and histological dedifferentiation can occur witho degrowth (metamorphosis of holometabolic insects).

Thus when the evidence is viewed as a whole, no room is left for doubt t a great deal of independence is possible between the fundamental processes ontogenesis. In considering what we mean by dependence we may distingu between existential dependence and dependence with regard to properties. the first case a part or process isolated from the organism would cease to ex altogether; in the second, it would continue to exist but with modified properti The fundamental processes of ontogenesis are evidently not existentially pendent on the integrity of the whole, but as in the case of the non-segmenti *Chaetopterus* egg, they do not take a wholly normal course when the integrati has been interfered with. It is as if each fundamental process represented layshaft which may or may not be in gear with the primary shaft, and the anim economy is obviously so constituted that more than one secondary gear can engaged with the primary shaft at one time. But in some cases, as we have se (p. 523), there is apparently a check on this flexibility, and if growth is in ge characteristic chemical activity cannot be. And *vice versa*. The engageme must be selective.

Lastly, what is the primary shaft? It is probably not identifiable with o chemical reaction, but may be defined as whatever reaction the cell can ca out which will provide it with the minimum amount of energy necessary maintain itself as a going concern in the physical world. In autotrophic bacte it may be the oxidation of ferrous carbonate to ferric hydroxide, or of metha to carbon dioxide and water; in metazoan cells it may be fermentation, t esterification and desmolysis of glucose. Whatever, under the worst enviro tal conditions, suffices for basal metabolism may be thought of as the primary it, or rather, the automotive unit to which the primary gearshaft is attached.

. Heterauxesis

hen a living organism grows, its parts, as is well known, do not necessarily v at exactly the same rate as the organism as a whole. Indeed a part of an nism which correctly reproduces the growth curve of the totality to which it ngs is the exception rather than the rule. "The form of an animal", as rcy Thompson wrote in 1916, "is determined by its specific rate of growth arious directions; accordingly the phenomenon of rate of growth deserves e studied as a necessary preliminary to the theoretical study of form, and hematically speaking, organic form itself appears to us as a function of time."* se facts have been well appreciated by the majority of morphologists, and od deal of information about the growth of parts has become available (cf. the maries of Scammon[1]; Scammon & Calkins; Fauré-Fremiet[1]; Hofmann; and dham (*CE*, p. 440)). If it remained somewhat un-coordinated, this was no bt due to the inadequacy of the terminological and conceptual technique at disposal. The work of J. S. Huxley[4] (see especially his book, *Problems of tive Growth*[9]) has, however, led the way towards a new order in this field.

21. Embryonic growth in general

efore proceeding further, a word must be said regarding the mathematical oach to embryonic growth in general. This was fairly fully reviewed up to in *CE*, Section 2, and since that time there have been a number of attempts ake further progress by applying mathematical analysis to growth phenoa. In general, however, there is very little of such progress to report.

he two most important books are those of Kostitzin and Rashevsky[3]. The ier gives an excellent account of the use of elementary mathematical tech- for the treatment of massed results; but the latter, with more originality, to apply a kind of mathematical physics to the behaviour of individual ic systems in certain specified circumstances and limitations.

ather ambitious treatment of embryonic growth has been given by Wetzel, ttempts to treat what he calls the "motion of growth" in terms of equations namics, especially by a modification of Lagrange's equations. From the of view of mechanics the use of Lagrange's equations is an analytical gem valid only in certain very clearly defined circumstances. For example, -called Lagrangian co-ordinates used must be capable of defining explicitly ms of themselves and of time the position of every element of the system. Vetzel, in using a single "generalised co-ordinate" to represent a single ism, is confusing the implicit interrelation between the various elements of ganised system which may be revealed as the *result* of calculation, with the citly expressible relations between the actual quantities used to represent elements. With its supply of constants adequate to fit almost any series of ts, Wetzel's treatment fails to give us a conviction of validity. The only

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oted elsewhere (p. 128), embryologists have been very slow to apply d'A. Thompson's inate distortions to embryonic development, but the first attempt to do this must be ed in the work of Richards & Riley. Unfortunately they have so far been almost exclusively ed with the changes in whole body form during the *later* stages of amphibian development.

precise correlation between experimental observations and the results of calc tion is found in the Gayda series of figures for growth and heat-productio the toad, but here inspection of the curves shows that there is not suffic goodness of fit to justify the precise mathematical system which Wetzel pounds.

Other workers have proposed exponential and logarithmic formulae, but sidering the fluidity and plasticity of the equations the fit of the curves leaves m to be desired. The most considerable volume of work of this kind is tha Backman and his school (e.g. Edlén); it cannot be said that their theore superstructure is in any way justified by their mathematics. The same rem apply to some extent to the work of Schmalhausen; Courtis; and v. Bertalan Evidence constituting strong criticism of these workers is presented by Lern who draws attention to periodic oscillations in the growth rate of the c embryo and rightly points out the over-simplification inherent in logarith plotting. The theory of the "master reaction" is attacked by Burton, w paper should be read by everyone interested in the subject, and Kavanag Richards and Richards[1] have demonstrated that the Crozier master reac equation is nothing but a function of the hyperbolic tangent. Teissier[7], valuable review, draws the following conclusion regarding the Robertson Crozier formulae: "L'exposé que nous venons de faire suffit amplemen montrer que les théories chimiques de la croissance n'ont donnés de cette dern que des schémas à peu près vides de tout contenu concret et parfaitement vérifiables."

The empirical formula of v. Hoesslin is in a class by itself, but none better for that.

The variabilities inherent in biological material have usually been insufficie appreciated by writers on the mathematics of embryonic growth. From criticism the writings of Duyff and Janisch are free, however, and the treatm they give to their data (on the chick embryo and insect respectively) is restra and reasonable.

The upshot of the matter is that the effort devoted to the mathematical ana of the growth of the whole body has been singularly lacking in fruitfulnes illumination. Perhaps this might have been appreciated long ago, had not ever-present temptation to prefer paper work to the mastery of difficult techni led us to forget the vast complexity of the organic systems of which we lik measure the wet weight. When we come, however, to the growth of p especially of chemical parts, as compared with the growth of the body as a wh we recognise more fully this complexity, and in the end, as the succee sections show, arrive at certain relations which seem to have a general si ficance.*

3·22. The theory of heterauxesis

There had been no lack of empirical equations, devised particularly in Ame relating the weight of parts to that of the whole by a series of linear equati in which many special constants were introduced. But in 1924 Huxley[4] demonstrated a simple and significant relation between the growth of a w organism and that of a part increasing or decreasing in relative size. It ready been used, in closely similar form, by Dubois in 1897 and Lapicque in 398 for the relative growth of the mammalian encephalon, but had remained dden in the literature. If x be the magnitude of the whole organism (as easured by some standard linear unit, or by its weight), and y the magnitude the differentially growing organ, the relation between them is

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* For the critical work embodied in the above paragraphs I am much indebted to two friend one a biologist and the other a mathematician, Dr Sydney Smith and the Rev. Christo Waddams.

$$y = bx^k \qquad \ldots\ldots(1),$$

here b and k are constants. The constant b is of little biological significance, nce it merely denotes the value of y when x is unity, i.e. the fraction of x which occupies when x is unity. It is called the "fractional coefficient". On the other nd, k has an important meaning, for it implies that over the range for which e formula holds, the ratio of the growth rate of the part to the growth rate of e whole remains constant.

Such a ratio was termed by Huxley a "constant differential growth ratio", d the whole process by Pézard "heterogony". The process may obviously be ther positive, if the relative size of the part increases with time (i.e. "grows ore quickly than the whole"), or negative, if the relative size diminishes e. "grows more slowly"). If it attains a very extreme degree, it may be called, Champy's phrase[2] "dysharmonic growth". Some authors, e.g. Teissier[1], at st preferred to use the word "dysharmony" instead of heterogony, but this s not acceptable since if we liken the organism to a piece of music, the growth all parts at the same rate would be "unison" and their growth at different tes "harmony", not "dysharmony". Later, after the term "heterogony" had me into general use, Huxley & Teissier proposed its replacement by "allo- etry" on the ground that it had previously for a long time been employed by x physiologists to denote a special type of reproductive cycle. But since terogony after all implied something inherent in the developmental plan of e organism, while allometry seems to refer rather to our metrical methods, eedham & Lerner suggested the word *Heterauxesis*,* with *Isauxesis*, *Bradyauxesis* *Tachyauxesis* for the three cases formerly called isogony, negative hetero- y, and positive heterogony. This terminology will be used here. At the same e, it may be convenient to have a terminology which distinguishes between erences *during* growth and *after* it. Comparisons may be made between anisms of the same group differing in age, size, weight, chemical composition, ; this is heterauxesis. But comparisons may also be made between organisms different groups (races, varieties, species, genera, and the like) differing in but of the same age, e.g. birth or adult maturity. Huxley, Needham & ner suggest that the word *Allomorphosis* should be reserved for this. In ny such cases the Huxley equation holds good; for example the relation ween egg size and bird weight (in his book), or the work of Hersh[2] on notheres, or that of Lerner, Gregory & Goss already mentioned (p. 426) on tathione, or that of Brody and Kibler on organ sizes. Since such differences se on account of heterauxesis, a word covering both this and allomorphosis y be useful; *Allometry* will do very well. The word allometry has been used a similar sense by Osborn, who spoke of the changes in bodily proportions urring during evolution as allometric changes. And the coordinate distortions l'Arcy Thompson would thus be said to represent allometric differences.

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* Due to my friend Dr A. L. Peck.

The formula given above may also be written

$$\log y = \log b + k \log x \qquad \ldots\ldots(2$$

from which it follows that any magnitudes obeying the equation will fall al straight lines if plotted on a double logarithmic grid. The appearance of a stra line on double log paper does not establish the sufficiency of the formula for case in question, since no purely graphical method could do so, but it aff strong evidence of its applicability. If the data were sufficiently good, each p could be verified by calculation. The logarithmic method of plotting, moreo emphasises an important point which is entirely obscured by the usual met of plotting on absolute scales, namely, the fact that growth is essentially concer with multiplication. Equal spaces on the logarithmic grid denote equal amou of multiplication, equal spaces on the ordinary scale denote equal additio Another great advantage of logarithmic plotting consists in the fact that embryonic development, where the numerical values on the axes cover such enormous range, it is impossible in any other way to view the course of devel ment as a whole in one and the same picture. For preliminary purposes, constant k can be read off from the slope of the straight line, for if a is the an it makes with the x axis

$$\tan a = k \qquad \ldots\ldots(3).$$

These methods were originally developed to deal with the phenomena arthropod limb growth, the growth of antlers in some mammals, and other simi morphological magnitudes.* But considerable scope exists for their applicati to magnitudes of a distinctively chemical nature. The metazoan organism, in passage from fertilisation to maturity, passes through a long succession of sta which are characterised just as much by changing chemical composition as changing morphological form. And just as we think of organs or structures parts of a morphological totality, so we may think of substances or groups substances as parts of a chemical totality. It is legitimate to enquire into t regularities which the constituents of this chemical totality exhibit during continuing increase in mass. Now there exist in the literature of chemi embryology and the biochemistry of growth a large number of sets of d regarding such entities as protein nitrogen, glycogen, calcium, etc., in a vari of embryos and animals throughout the phyla. These may be considered fro the point of view of heterauxesis. The constant k is here of great value, for permits us to compare quantitatively the percentage relationships of any su stance in any developing organism. A family of curves with meaningless sha relating a chemical entity to age for several embryos may become a series straight lines of the same slope when the entity is logarithmically plotted agai the totality. This is in fact the case, and arises because the complicating factor time is not explicit in such graphs. The constant b, on the other hand, though fixes the percentage composition of the body at unity, is not of great use, sin time at which unity is reached occurs at very different points in the life cycle ng to the great variation in the sizes of animals, and this is still true no matter at scale is taken.

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* A specific rate of relative growth, expressible by a heterauxetic constant, may be regarded the "fate" of an organ just as much as its characteristic histology. Hence we may speak of t determination of a growth-rate, as in the considerable body of work reviewed in Section 2·6

Teissier[1] in 1931 was the first to apply heterauxetic theory to the chemical elopment of an organism. Taking as experimental animals the larvae of the ilworm and the waxmoth, he studied the increase in a number of chemical ities, such as water, fat, ash, phosphorus, etc., and found that in all cases xley's relation was obeyed as the totality grew. Some of his results will sently be considered in more detail. The behaviour of the same substance in erent classes of animals was, however, left over until in the following year, paper mainly concerned with the evaluation of k for all the constituents of chick embryo, I drew attention[9,10] to the rather close correspondence which be exhibited by the relative "growth" of a given chemical entity in very erent animals both in embryonic and later stages.*

Before going on to the experimental, or rather, observational data, reference st be made to the logical status of Huxley's equation. According to the theory imensions, the dimensions on the two sides of the relation must be equivalent. his case,

$$y = bx^k \qquad \ldots\ldots(1),$$

are only so when k is 1, which is comparatively rare. Ordinarily, since x is ass, x^k cannot be. The equation, therefore, has no true physical meaning, is to say, no new concept can be deduced from it as it stands, in the sense the concept of acceleration arises from the relation

$$Mf = a \qquad \ldots\ldots(4),$$

veen mass, force, and acceleration. The constant k is enigmatic without her elucidation, and cannot itself provide any theory of growth. The equation ntially gives us a good technical method for the examination of curves.

he point at issue may be made clearer by means of an analogy. The theory mensions is not satisfied in the relation

$$\Delta \propto c \qquad \ldots\ldots(5),$$

e Δ is osmotic pressure and c is the concentration of a solute. Accordingly, the implications in the relation are made explicit by considerations due to ic theory, the relation has no true physical meaning. A relation similar to heterauxesis relation occurs in magnetism. If a mass of steel be carried ugh a cycle of flux from a maximum induction B to an equal negative and again, the energy lost in such a cycle (Joules), per cubic centimetre of erial, is

$$J = kf(B) \qquad \ldots\ldots(6).$$

v $f(B)$ has the same shape for all steel alloys, which differ solely in the value . Further, over a long range of B, $f(B) = c\,.\,B^{1\cdot 6}$. Hence over that range

$$J = \eta B^{1\cdot 6} \qquad \ldots\ldots(7).$$

e idea of chemical heterauxesis has also been extended to plant development (Meunier[1]; & Williams). Refined mathematical methods for detecting genetic differences in k are ibed by Schmalhausen; Feldstein & Hersh and by Lerner[1].

Here there is no theoretical explanation of the exponent, and η is a cons
varying with the alloy. The formula conveniently expresses relative losses,
a "magnetic theory" could not be founded on it, for it is purely an empiri
verifiable numerical relation. Ultimately it arises from the fact that work
to be done to orient the particles of the iron. Similarly, in the heterau
equation for chemical entities, the ultimate explanation might be in term
colloidal stability, but nothing can be premised concerning it from the equa
in the absence of further experiment and observation.*

Attempts have already been made to offer a physical interpretation of
heterauxesis equation. Robb suggested that the distribution of "building-sto
molecules between circulating blood and tissue cells proceeds in a similar
to the distribution of solute molecules between two phases. Each organ w
thus have a partition coefficient of its own for any given substance, and w
grow more rapidly or more slowly than the body as a whole according to
value of this coefficient. Robb regarded the constant k as a measure of
concentration or activity of a substance governing the partition (i.e. capabl
altering the partition coefficient), in so far as it was present in the orga
greater or less concentration than in the body as a whole. He also modified
Huxley equation by the insertion of a term, c, to represent the amounts of i
non-growing substance present in the organ rudiment; thus

$$y = bx^k + c \qquad \ldots\ldots(8$$

The requirements of the theory of dimensions do not seem to be any be
satisfied here than in the original equation. This does not mean that the th
of partition coefficients is inapplicable, only that it does not follow from
empirical regularities so far found to hold. Moreover, it will hardly do w
chemical entities are concerned, for something more subtle is required to
the balance between them. How far the introduction of a correction for
growing constituents helps is not clear. To this it may be added that Meda
later demonstrated the validity of the Huxley equation for long and short
when an explant is made to grow in an artificial field of growth-inhibitory s
stance.

The second attempt to find a physical basis for the heterauxetic relation is
different from that of Robb. O. Glaser & Child noticed that the mathematical se
which describes the stacking of hexoctahedra around a central hexoctahedro
successive shells is related to the heterauxetic formula. The orthic hexoctahed
is bounded by six non-contiguous squares and eight hexagons; it is the ideal f
taken up by compressed adjacent spheres of equal size so that the mate
aggregates with no waste spaces, as may be shown experimentally (Matzk
Polyhedra of this type are actually visible microscopically in yolk, caterp
tissues, etc. The mathematical relationship arises because the Marvin se
which describes the stacking of the polyhedra, has the form

$$\log S_n = 3\log(2n+1) - 0{\cdot}301, \qquad \ldots\ldots(9$$

where S_n is the number of individuals stacked in n layers about a central u
This is formally identical with the growth formula of Glaser:

$$\log w = k\log(2t+1) + c, \qquad \ldots\ldots(10)$$

---

* But see the discussion of Lumer. The relations between the heterauxetic equation and
sigmoid growth curve have been discussed by Bernstein[1].

† Cf. the description of d'Arcy Thompson (*Growth and Form*, pp. 336 ff.). The connec
of spiral growth with another series, the Fibonacci series, is well known.

ere w is the weight of the totality or entity which is growing, t time, and k
l c constants (see his full discussion of this). And therefore the stacking series
uld replace either organic correlative in the heterauxetic equation, and this,
ording to Glaser & Child, it will do. It remains difficult, however, to under-
nd why chemical substances should obey this law, especially where molecules
ich smaller than the hypothetical intracellular polyhedra are concerned. As
it is too early to appraise fully the suggestion of Glaser & Child, which must
left for further investigation.

So far practically all cases of chemical "growth" give linear plots on the double
arithmic grid. O. Richards[1], however, has drawn attention to the fact that this
ot always so in the case of morphological entities, where sometimes k seems
be continually changing or oscillating widely about a mean. He sounds a
tified caution in accepting any theory of heterauxesis.

Not all the sets of data for the relative growth of chemical constituents are
resentable, however, by *single* linear plots; frequently two or even three
arate straight lines may be found necessary. The breaks between these
cessive phases obviously indicate a change in one or other of the constants in
equation. If k changes, the slope of the line will alter; if b changes, there will
a period of nil growth of the part, followed by the resumption of growth at
same rate as before. These possibilities are exemplified in Fig. 291, which
ws the water-soluble non-protein nitrogen of the chick embryo (*CE*, p. 1075)
tted against the embryo's dry weight, and the water of the waxmoth larva
eissier[1]) plotted against the wet weight of the larva. The former entity grows
h tachyauxesis ($k = 1{\cdot}69$) up to the 6th day of development, when the embryo
ighs approximately 15 mgm. (dry), after which it suddenly becomes brady-
etic ($k = 0{\cdot}83$). The latter entity (water in the waxmoth) grows throughout
whole period with a k of 0·96, but between the weights of 60 and 100 mgm.
et) there is a period of adjustment during which b changes from 0·0075 to
·55.

the results of Teissier[1] for the insect larvae are compared with those of
dham[10] for the chick embryo, it is at once noticeable that changes of the first
(changes in k) are much more characteristic of the embryonic material than
e of the second type, and *vice versa*, although a few examples of the opposite
elation can be found. Any interpretation of this fact might as yet be pre-
ure, but it may not unreasonably be connected with the circumstance that
larval insect growth is much less accompanied by profound changes of
phological differentiation than is the growth of the chick embryo. Further
mples are needed to test this hypothesis. For the present purpose, however,
ussion will be mainly confined to those cases where the data exhibit no breaks,
changes in either b or k. This is not because the breaks may not be perfectly
l, but because greater certainty of comparison between different organisms
be attained by the use of data which do not show them. For example, the
ium in the chick embryo has been determined by several investigators
erences and plots in Needham[10]), some of whose series give breaks of k. But
n the entire mass of data is plotted without distinction of investigator, it is

found to lie with equal deviation on both sides of a straight line from the sma to the very largest embryos. This suggests that with further work, many of changes in k hitherto recorded may turn out to be illusory. Here, theref emphasis will only be laid on sets of data giving the unbroken logarith relationship, and on its similarity between different animals.

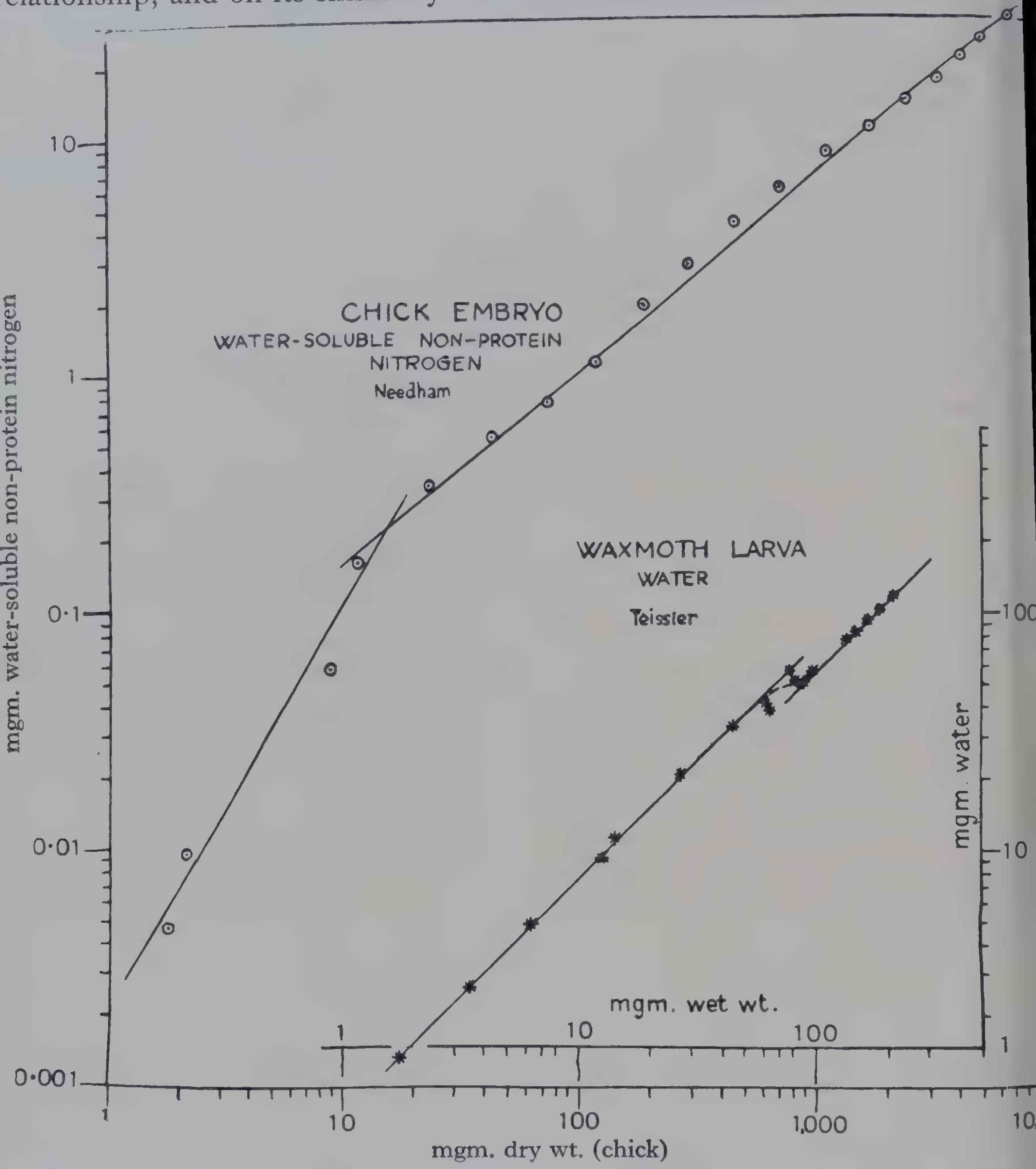


Fig. 291. Examples of change in b, and change in k: for the former; water in the waxmoth (Teissier): for the latter; non-protein nitrogen in the chick embryo (Needham).

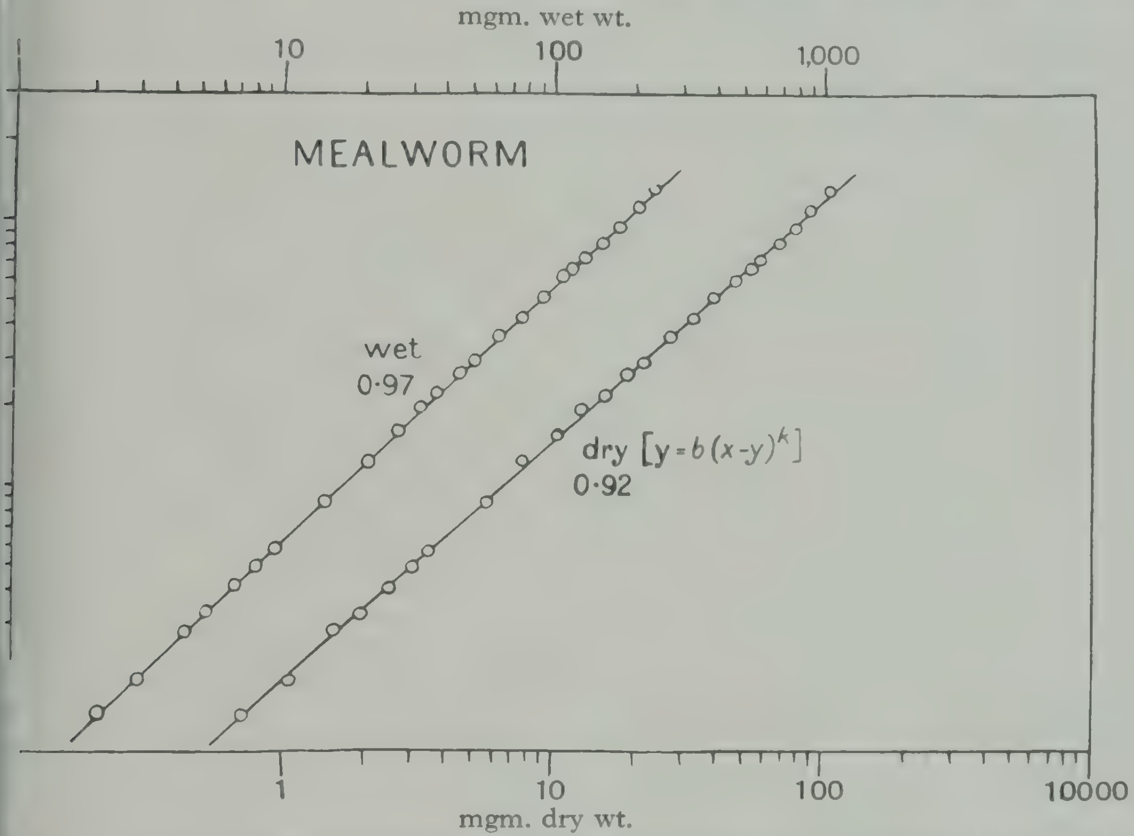
It may be mentioned, however, that certain investigators have believed in existence of well-defined critical points at which changes occur in the he auxetic constants of numerous groups of chemical substances. Thus Teissier[5,6] located two such critical points in the development of the chick embryo, on 1·5 gm. and the other at 12 gm. (wet weight) or 9 and 15 days respectiv Variations in k, which may be either in the tachyauxetic or bradyauxetic direct are said to occur at either or both of these points, in the data for purine nitro

-protein nitrogen, lipid P, and total fat. Meunier[2] added potassium. But it is doubtful whether all the available data of sufficient reliability support these lusions, and the matter requires much further investigation.

the following graphs it will be seen that in all cases the mass of the chemical ty is plotted against the mass of the chemical totality. Although this is quite al, it implies that in the case of a part growing tachyauxetically, i.e. faster the whole, a time must eventually come when the mass of the part equals of the whole, and finally exceeds it. This unfortunate state of affairs is ded by writing the equation

$$y = b(x-y)^k \qquad \ldots\ldots(11),$$

plotting the part against the remainder of the whole rather than the whole f. This was the method adopted by Huxley[9] in the first instance, who defined



292. Comparison of the relation of water in the mealworm larva to wet and dry weight (Teissier).

ordingly, although the graphs in his book do not seem to be always con- t with it. Teissier[1], on the other hand, used the formula (1), and its application emical heterogony will be continued here. The question is somewhat mic, since growth is not infinite, and a point imagined as travelling along f the straight lines in the succeeding figures would slow down and finally at the adult condition long before the consequences of the convention here ted became serious. Moreover, the difference in k is not very marked for wo conventions. In the case of a very small part it is quite inappreciable, even in that of a very large part, such as the water-content of the body, it nparatively small. Fig. 292 shows the water in the body of the mealworm plotted against the wet weight (x) and the dry weight ($x-y$) respectively. e former case k is 0·97, in the latter case 0·92.

A word should here be said concerning the reproducibility of grap
chemical heterauxesis. It seems to be satisfactory. In Fig. 293 the soli
represents the relation of dry weight to wet weight in the chick embr
determined from the averaged data of thirteen separate investigators (see Fi
in *CE*). Points represent the data subsequently reported by Saccardi & L
who appear to have been unaware of the work of their predecessors, ar
Penquite. It will be seen that over the greater part of the range the agree
is excellent, although in the earliest stages, when the embryos are very sma
points of the Italian workers show a slight divergence.

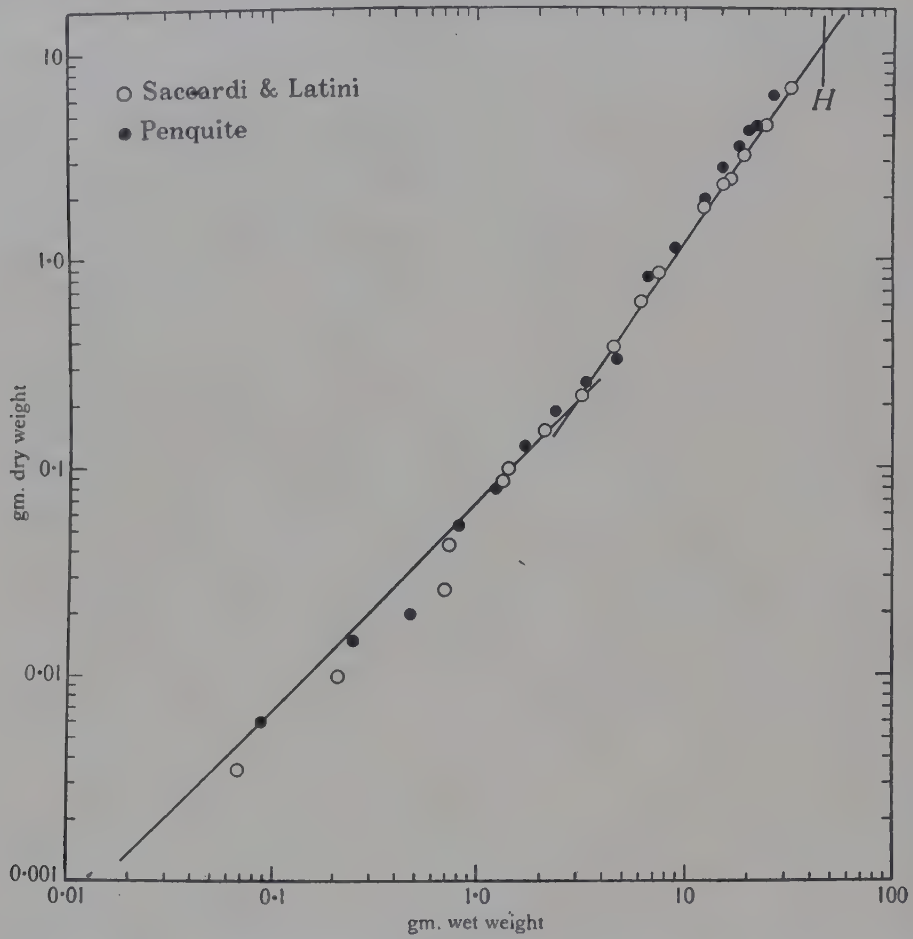


Fig. 293. Relation of dry to wet weight in the chick embryo; confirmation of earlier wor
Saccardi & Latini, and by Penquite.

3·23. Chemical heterauxesis

We may now go on to specific examples. It may be convenient first to co
water-content. Fig. 293 showed that during the first period in the developm
the chick embryo, the increase in solids is practically isauxetic, and duri
second period strongly tachyauxetic ($k = 0{\cdot}93$–$1{\cdot}06$, then $1{\cdot}43$–$1{\cdot}48$). This
a more accurate way of stating the well-established fact that the chick e
becomes drier as it develops (see *CE*, p. 871). For organisms other than
numerous sets of data exist covering in some cases the whole life cycle, in
nly pre-natal or only post-natal life. A good many of these are plotted in F
double logarithmic grid, and the references and constants are co

Table 29. The points of birth or hatching are suitably marked on the chart in h case (Needham[16]).

t is at once obvious that within any one group of animals, there is a marked ilarity of slope, that is to say, they get drier at the same absolute rate. The mmals, for instance, all show a *k* closely approximating to 1·23, and it is narkable that over the whole growth period of man (from 1 to 66,000 gm. t wt.) this relationship holds good. Again, the three researches on teleostean es give a *k* of unity, indicating that over the period studied (from some time ore hatching until the end of the free-swimming larval period) the tissues are drying at all. The same statement applies to the woodlouse, which, however, only been measured (and that some considerable time ago) after hatching. tical points, where *k* changes, appear both in the chick, and in some of the chian fishes, which show the same change from isauxetic growth of the dry ight to marked tachyauxetic growth. On the whole, it may be concluded that weight never shows bradyauxesis, that is to say, all organisms dry up with , and that within one group, the absolute rate at which this process takes place, very uniform indeed. In Table 29 the ranges of *k* are given as well as the rage values. The birds alone show a wide range, but this may be explained the existence of two separate phases, and the restricted nature of some of the s of data available. The range of all *k*'s for all organisms is only 0·27.

n those cases where no drying up of the organism is taking place, we have not yet sufficient knowledge of the dry weight throughout the life cycle to say ether the process of dehydration has finished or whether it has not yet begun. is tempting to relate the concurrence of evidence for the isauxesis of the eostean fishes, with the views of Bidder on the absence of adult size in some uatic organisms, and the possible absence of death from senility. Although ice and carp, he suggests, may continue to grow until many times the age of ir sexual maturity, and although we have no reason to suppose that they ever except by violence, the same could not be true of swiftly moving terrestrial tures. These must maintain a relation between their weight and the cross-ional area of their bones and muscles, necessitating a definite adult size, e, and habit. Old age and death by senility became the necessary fate for nals only when they left the water and attempted swiftness and tallness in edium 1/800th of their own specific gravity. On the other hand, there is at sent no obvious reason why the tissues of selachian fishes should dehydrate growth, while those of teleosts do not. The subject merits closer attention n it has yet received.

he next example which may be taken is that of fat. Fig. 295 gives the appear-e of the data for rat, chick, and mealworm when plotted on the double arithmic grid (data of Chanutin; H. A. Murray; Cahn & Bonot; Romanov[5]; and issier[1]). For the insect and the bird, in spite of the great differences in the solute weights concerned (e.g. at hatching or birth, mealworm = 3 mgm. dry , chick = 7000 mgm. dry wt., rat = 660 mgm.), the correspondence is very d; the rat shows slightly more tachyauxesis. The values of *k*, together with ir range of variation, are shown in Table 30. In all cases, the auxesis is

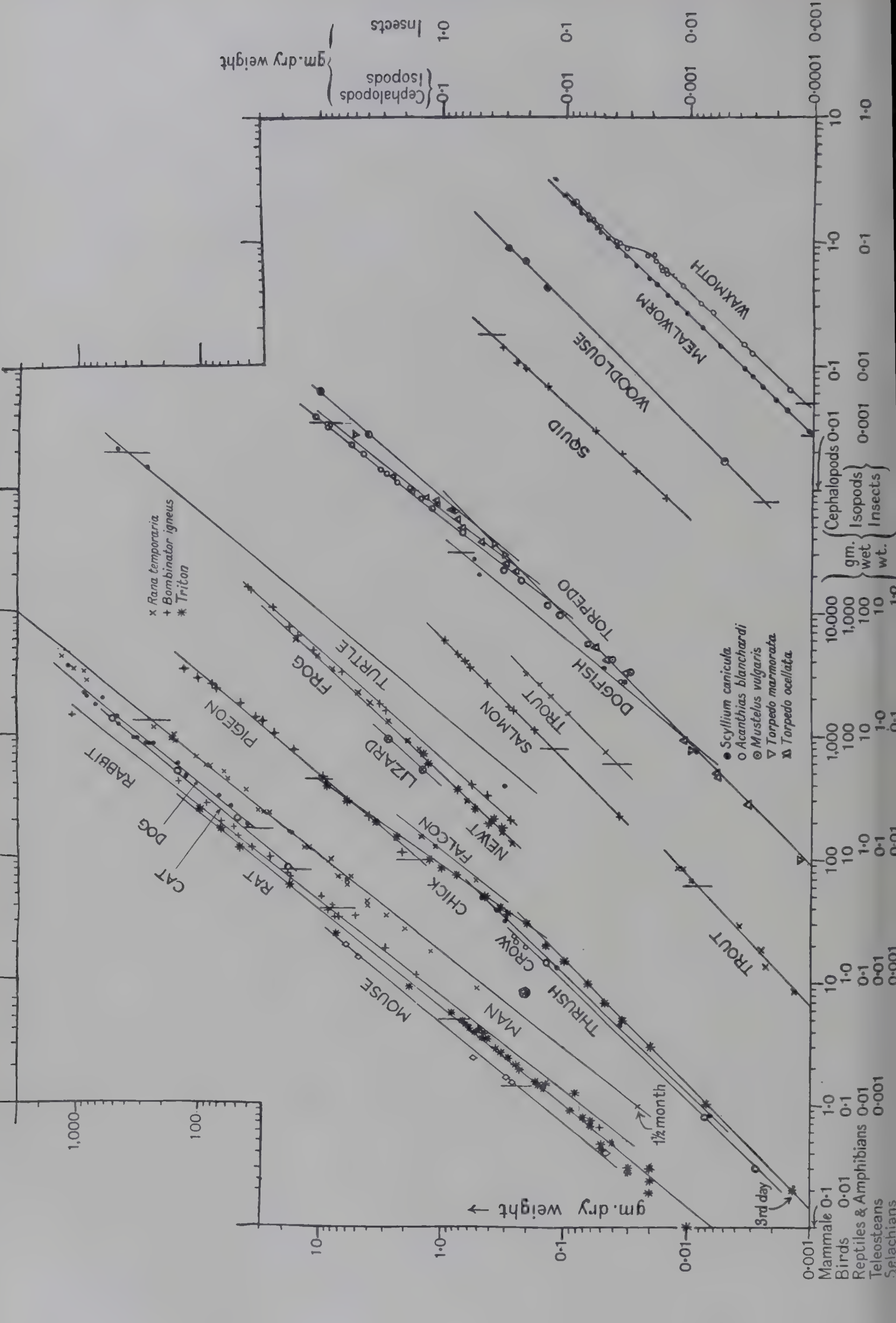

gm. dry weight
Cephalopods
Isopods
Insects
1·0
0·1
0·01
0·001
0·0001
WAXMOTH
MEALWORM
WOODLOUSE
SQUID
TORPEDO
DOGFISH
TROUT
SALMON
TROUT
● Scyllium canicula
○ Acanthias blanchardi
⊙ Mustelus vulgaris
▽ Torpedo marmorata
⊠ Torpedo ocellata
× Rana temporaria
+ Bombinator igneus
✱ Triton
TURTLE
FROG
LIZARD
NEWT
PIGEON
FALCON
CHICK
CROW
THRUSH
MAN
MOUSE
RAT
CAT
DOG
RABBIT
1½ month
3rd day
← gm. dry weight
1,000
100
10
1·0
0·1
0·01
Mammals
Birds
Reptiles & Amphibians
Teleosteans
Selachians
gm. wet wt.
10,000
1,000
100
10
1·0
0·1
0·01
0·001

Table 29. *Dry weight as function of wet weight*

[Numbers in brackets refer to the notes below the table; superscript numbers are bibliographical.]

Organism	Investigators	Period	k	Range of k	Average k
1)					
mo sapiens)	Michel; Fehling; Brubacher; Camerer & Söldner; Langstein & Edelstein; Sommerfeld; Moleschott; Bischoff; collected by H. Aron; also Schmitz and von Bezold	Whole life	1·26	0·09	1·23
epus cuniculus)	Fehling; Schkarin; Friedenthal; Steinitz; collected by H. Aron	Whole life	1·28		
nis vulgaris)	Thomas; Gerhardtz; Orgler; Eckert; collected by H. Aron	Whole life	1·24		
vulgaris)	K. Thomas	Post-natal life	1·21		
norvegicus)	Lowrey; Donaldson; collected by Donaldson, also Gonzalez	Whole life	1·23		
	Hamilton & Dewar	Whole life (0·072 gm. to 435 gm. wet wt.)	1·19		
Mus vulgaris)	von Bezold	Whole life	1·21		
mo sapiens)	Iob & Swanson[1]; Hamilton	Embryonic life	1·27	—	—
	Givens & Macy	Embryonic life	1·17	—	—
norvegicus)	Roche & Garcia	Post-natal life (−25 days)	1·33	—	—
scrofa)	Wilkerson & Gortner[2] (2)	Embryonic life	1·07	—	—
llus domesticus)	Murray; Romanov; Byerly; Mankin; collected in *CE*	Embryonic life	1·42	0·39	1·19
Meleagris americana)	Martin & Insko[1]; Scott & Hughes	Embryonic life	1·20		
Columba livia)	Kaufman[1]	Post-embryonic life	1·19		
Turdus merula)	Groebbels[2]	Embryonic life	1·03		
rvus corone)	Groebbels[2]	Embryonic life	1·04		
uteo buteo)	Groebbels[2]	Embryonic life	1·28		
Lacerta viridis)	von Bezold	Post-embryonic life	1·11	0·09	1·15
Thalassochelys corticata)	Karashima	Embryonic life	1·20		
na temporaria)	von Bezold	Post-larval life	1·07	0·08	1·09
mbinator igneus)	von Bezold	Post-larval life	1·15		
Triton cristatus)	von Bezold	Post-larval life	1·07		
N FISHES					
(*Salmo salar*)	Hayes[1]	Embryonic and larval life	1·00	0·01	1·00
Salmo fario)	Gray[1]	Embryonic and larval life	1·00		
	Kronfeld & Scheminsky	Embryonic and larval life	1·00		
prinus carpio)	Nowak	Larval and post-larval life	1·01		
FISHES					
Scyllium canicula)	Ranzi[12]	Embryonic life	1·21	0·12	1·27
(*Torpedo ocellata*)	Ranzi[12]	Embryonic life	1·00 then 1·33		
(*Torpedo marmorata*)	Ranzi[12]	Embryonic life	1·00 then 1·32		
Acanthias blanchardi)	Ranzi[12]	Embryonic life	1·00 then 1·29		
Mustelus vulgaris)	Ranzi[12]	Embryonic life	1·00 then 1·23		
m (*Tenebrio molitor*)	Teissier[1]	Larval life	1·04	0·01	1·035
(*Galleria mellonella*)	Teissier[1]	Larval life	1·03		
(terrestrial)					
se (*Oniscus murarius*)	von Bezold	Post-larval life	1·00	—	1·00
le (*Littorina irrorata*)	Newcombe & Gould	Post-larval life	0·95	—	—
Sepia officinalis)	Ranzi[6]	Embryonic life	1·09	—	—

Notes to Table 29

data of Esskuchen[2] for the cow, though interesting, are excluded as lacking a sufficient number of separate points.
mised data for this animal have not yet become available, nor have those of Scammon[2] for man as far as I can ascertain,
entioned in a preliminary note ten years ago. All individual organs show a desiccation, similar to that of the body as
e e.g. Duyff's data on the chick embryo's organs), and so does the blood; thus at 3 months' foetal life in man the blood
7·8 % water and at birth only 67·3 % (Takakusu, Kuroda & K. N. Li; Kuroda).
hese authors measured dry weight after acetone extraction their data are not comparable with other sets.
ery early embryonic life birds often show isauxesis or even bradyauxesis of dry weight (e.g. *k* 1·00 for the chick during the
and 0·67 for the turkey). Cf. the initial rise of the curve in *CE*, Fig. 220. But as not all sets of data show it I am inclined
may possibly be due to technical errors in the handling of very small embryos. There must of course be an upper
etness" probably represented by the gastrula itself, the dry weight of which no one has yet succeeded in measuring.

Table 30. *Chemical heterauxesis in various organisms*

[N.B. Values for entities not listed in this table may be found in Needham [10,16].]

Fig.	Chemical substance or group of substances	Animal	Investigators	Period	*k*	Ran of *k*
295	Fat (to dry wt.)	Waxmoth	Teissier[1]	Larval	1·32	0·03
		Mealworm	Teissier[1]	Larval	1·07	
		Chick	H. A. Murray; Cahn & Bonot; Romanov[5]	Embryonic	1·06	
		Rat	Chanutin	Post-natal	1·09	
		Man	Iob & Swanson[1]	Embryonic	1·07(1)	—
305	Fat (to wet wt.)	Chick	Romanov & Faber	Embryonic		
				32° C.	1·84	—
				34° C.	1·85	—
				36° C.	1·77	—
				38° C	1·79	—
291	Non-protein nitrogen (to dry wt.)	Mealworm	Teissier[1]	Larval	0·77	—
		Chick	Needham[2]	Embryonic	0·83	—
—	Purine nitrogen (to total N)	Chick	Lebreton & Schaeffer	Embryonic	0·72	0·1
		Pig	Lebreton & Schaeffer	Embryonic	0·81	
		Mouse	Lebreton & Schaeffer	Embryonic	0·62	
296	Creatine (to wet wt.)	Chick	J. Mellanby	Embryonic	1·12	0·02
		Rat	Chanutin	Post-natal	1·10	
244	Glutathione (to wet wt.)	Chick	H. A. Murray	Embryonic	0·84	0·06
			Kamiya	Embryonic	0·90	
			Yaoi	Embryonic	0·87	
			Cahn & Bonot	Embryonic	0·88	
		Rat	Thompson & Voegtlin	Whole life	0·84	0·01
		Pig	Wilkerson & Gortner	Embryonic	0·85	
297	Glycogen (to wet wt.)	Chick	Vladimirov & Danilina	Embryonic	1·42	0·07
		Pig	Mendel & Leavenworth[1]	Embryonic	1·35	
		Rabbit	Lochhead & Cramer	Embryonic	1·35	
298	Liver glycogen (to wet wt. of liver)	Chick	Vladimirov[1]	Embryonic	1·99	0·03
		Rabbit	Lochhead & Cramer	Embryonic	1·96	
299	Total ash(2) (to dry wt.)	Mealworm	Teissier[1]	Larval	0·82	0·14
		Squid	Ranzi[6]	Embryonic	0·86	
		Dogfishes	Ranzi:[11]			
			Scyllium canicula	Embryonic	0·96	
			Torpedo marmorata	Embryonic	0·96	
			Torpedo ocellata	Embryonic	0·86	
			Mustelus vulgaris	Embryonic	0·85	
		Carp	Nowak	Larval and post-larval	0·96	
		Chick	H. A. Murray	Embryonic	0·89	
			Romanov[2]	Embryonic	0·89	
			Bishop[2]	Embryonic	0·85	
			Saccardi & Latini	Embryonic	0·94	
			Penquite	Embryonic	0·94	
		Turkey	Martin & Insko[1]	Embryonic	0·93	
		Rat	Chanutin	Post-natal	0·95	
			Buckner & Peter	Post-natal	0·93	
		Man	Givens & Macy	Embryonic	0·96(3)	—
			Iob & Swanson[1]	Embryonic	0·99(4)	—
—	Sodium (to dry wt.)	Chick	Mankin[3]	Embryonic	0·69(5)	—
		Man	Iob & Swanson[1]	Embryonic	0·70	—
—	Potassium (to dry wt.)	Trout	Leulier & Paulant	Embryonic and larval	0·90	—
		Chick	Leulier & Paulant	Embryonic	0·96(6)	—
			Mankin[3]		0·96	—
		Man	Iob & Swanson[1]	Embryonic	0·80	—
—	Magnesium (to dry wt.)	Rat	Greenberg & Tufts	Post-natal (to 18 wks)	1·20	—
		Man	Givens & Macy	Embryonic	1·09	—
			Iob & Swanson[1]	Embryonic	0·90	—
300	Calcium (to dry wt.)	Trout	McCay, Tunison, Crowell & Paul	Larval	1·16	0·08
		Chick	Mankin[3]; Plimmer & Lowndes; Romanov[1,2]	Embryonic	1·21	
		Turkey	Insko & Lyons[1]; Martin & Insko[1]	Embryonic	1·15	
		Rat	Sherman & McLeod	Post-natal	1·13	
		Man	Schmitz	Embryonic	1·20	
			Givens & Macy	Embryonic	1·15	
			Iob & Swanson[1]	Embryonic	1·15(7)	
—	Chloride (to dry wt.)	Chick	Mankin[2]	Embryonic	0·65	—
			K. Yamada[2]	Embryonic	0·78	—
		Man	Iob & Swanson[1]	Embryonic	0·71	—

Table 30 (*cont.*)

mical substance or up of substances	Animal	Investigators	Period	k	Range of k	Average k
phosphorus (to wt.)	Mealworm	Teissier[1]	Larval	0·97		
	Waxmoth	Teissier[1]	Larval	0·95		
	Silkworm	Akao[3]	Larval	0·99		
	Trout	McCay, Tunison, Crowell & Paul	Larval	1·00		
	Turkey	Martin & Insko[1]	Embryonic	0·96		
	Rat	Buckner & Peter; Sherman & Quinn	Post-natal	1·00	0·07(8)	0·97
		Roche & Garcia	Post-natal (to 25 days)	0·86		
	Man	Iob & Swanson[1]	Embryonic	0·93		
n phosphorus (to wt.)	Mealworm	Teissier[1]	Larval	0·80	—	—
	Chick	Cahn & Bonot	Embryonic	0·78	—	—
		Kugler	Embryonic	0·74	—	—
ein phosphorus (9) dry wt.)	Mealworm	Teissier[1]	Larval	0·80	—	—
(10) (to dry wt.)	Chick	McFarlane & Milne; Szejnman-Rosenberg	Embryonic	0·87	—	—
		Szejnman-Rosenberg	Emb. membranes	1·13	—	—
er (to dry wt.)	Chick	McFarlane & Milne	Embryonic	0·85	—	—
(to wet wt.)	Chick	Sümegi	Embryonic	0·96	—	—
(to dry wt.)	Chick	Bishop[1,3,4]	Embryonic			
			High	0·93	—	—
			Low	0·93	—	—

Notes to Table 30

50 gm. dry wt., $k = 1·82$ afterwards.
r data could be calculated from Cox & Imboden and Kozhukhar.
set of data there are indications of a preliminary early period of tachyauxesis ($k = 1·66$ below 3 gm. dry embryo wt.).
plies to Nowak's data on the carp (1·28 before 0·04 gm. dry alevin wt.).
gain there are indications of early tachyauxesis ($k = 1·54$ below 50 gm. dry embryo wt.).
speaking 0·91 before the 14th day and 0·47 afterwards.
0·6 gm. dry wt.; above that point the bradyauxesis is more marked ($k = 0·63$).
are faint indications here of a late period of bradyauxesis ($k = 0·85$ after 300 gm. dry wt.).
ng the figure from Roche & Garcia's data, which cover only a short period.
figures for purine nitrogen higher in the table; see also pp. 632 ff.
lata of Katsunuma & Nakamura and of Kojima are omitted as remarkably aberrant. Akao[4] has published figures for
ts as well as iron from which heterauxetic constants for the silkworm could be calculated.

ive, that is to say, fat is increasingly present in the body with growth. Other ts of this phenomenon are often met with, e.g. the development of mutton ty in the sheep (Hammond & Appleton); the chemical development of the malian brain (to be referred to later); and the fact that the calorific value of loping tissues (H. A. Murray; Penionskevitch & Schechtman on the chick; on the fish *Silurus glanis*) increases tachyauxetically ($k = 1·03$ for the chick, or the mealworm).

xt, two tissue extractives, creatine and glutathione, are illustrated in Figs. nd 244. The tachyauxesis of creatine in the chick shown by J. Mellanby's tunately rather restricted) data is exactly paralleled by that of the same nce in the rat according to Chanutin. Similarly, the marked bradyauxesis tathione is identical for the rat and the pig (data of Thompson & Voegtlin Vilkerson & Gortner respectively) and also the chick embryo (see Fig. 244 tion 2·68).* Creatine, then, "grows" more rapidly than the whole body, hione more slowly. The range covered by the data in Table 30 is very wide, case of the rat from 0·2 to 200 gm. wet weight, in the case of the pig from 1000 gm. wet weight. The well-known decline in nucleoplasmic ratio is in the bradyauxetic constants for nuclein nitrogen and phosphorus.

vcogen is a substance which has a rather special relation to embryonic Until the liver is sufficiently developed to store it, it is laid down in

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re have been numerous other sets of data for glutathione (e.g. in the chick embryo, Tateishi[1]; ; Castagna & Talenti) but I have found them either inaccessible or useless.

the extra-embryonic structures (mammalian placenta, avian yolk-sac) w perform the functions of a transitory liver (see *CE*, Section 8·5). Neverthe there is remarkable regularity between different animals in the rates at w the substance increases in the embryonic body itself. Fig. 297 shows the dat the chick, rabbit and pig, all of which give a k in the near neighbourhood of Even when we consider the accumulation of glycogen in the embryonic li we find that $k = 1{\cdot}97$ would hold for both chick and rabbit (Fig. 298)—a markable circumstance (when we remember that structures so different as the a

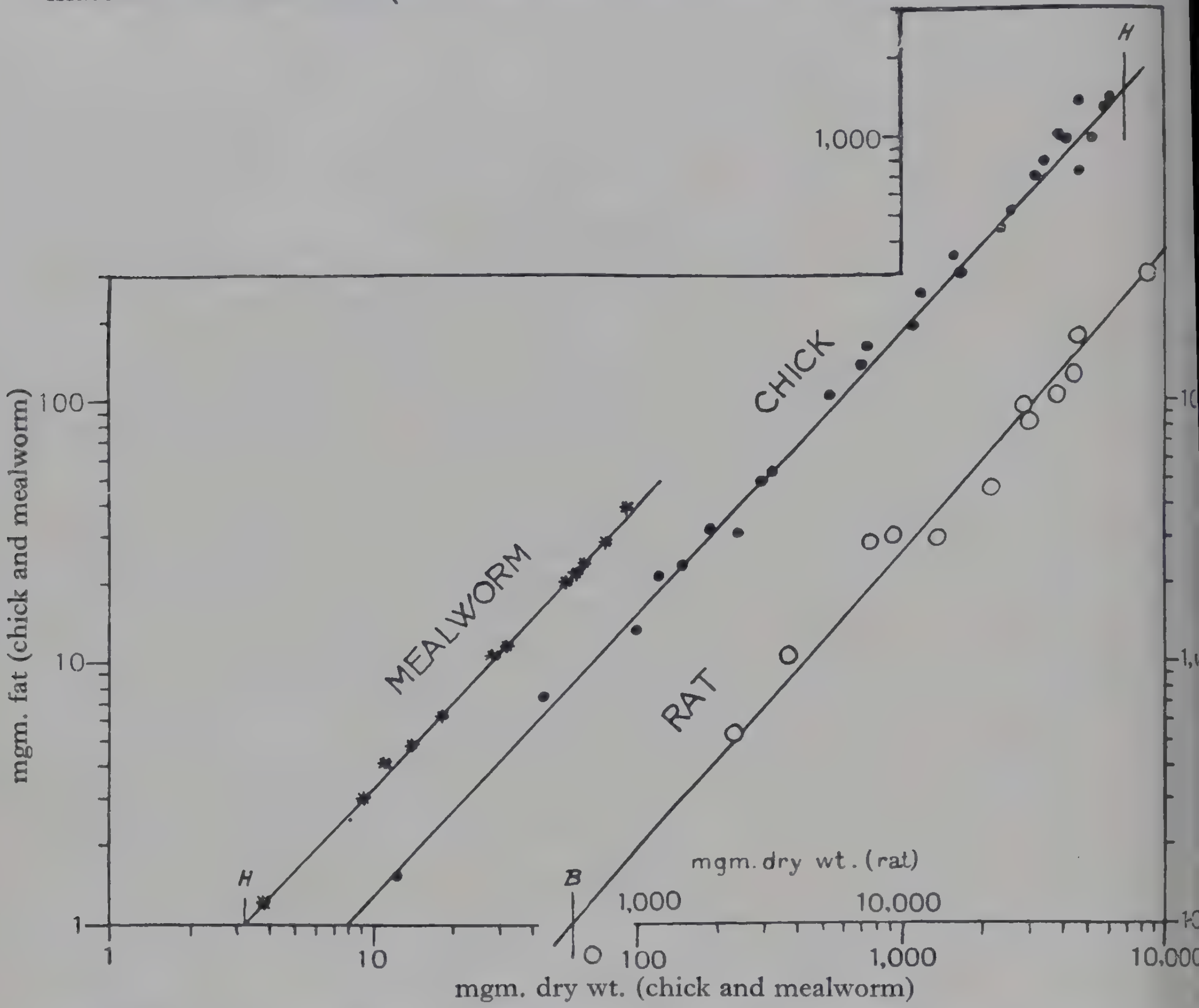
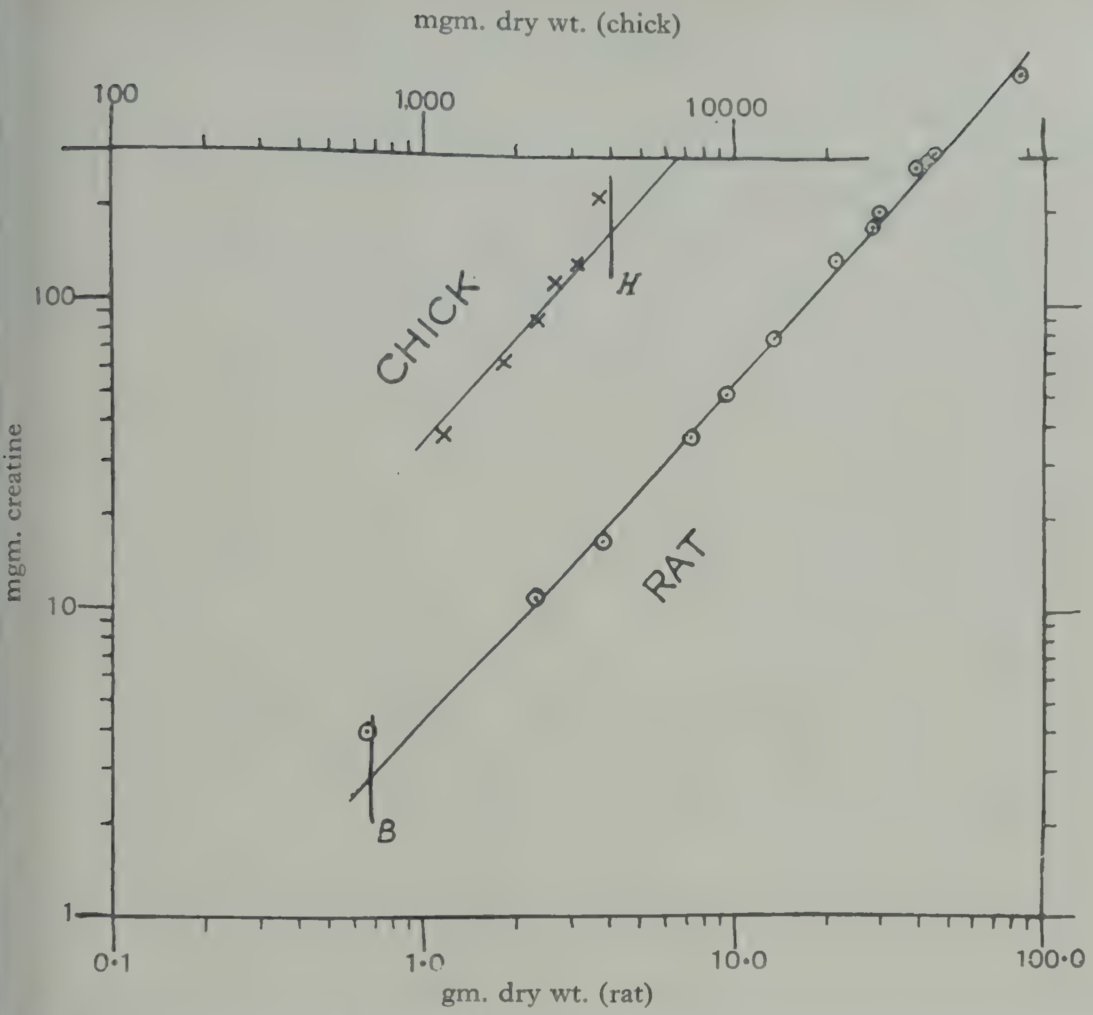


Fig. 295. Fat in the mealworm (larval period; Teissier), the chick (embryonic period; Mur Cahn & Bonot; and Romanov), and the rat (post-natal life; Chanutin).

yolk-sac and the rodent placenta are handing over glycogen to the foetal liver a serving as temporary depots themselves). It is interesting that after the chan over point the heterauxesis follows a different course, i.e. the process is strictly reversible (see Needham[10]).

The next graph (Fig. 299) is a particularly interesting one. The total ash of body is plotted against the dry weight for the chick, rat, squid, mealworm, several selachian fishes. The chick, the squid and the fishes are all embryo the mealworm is larval, and the rat is post-natal. There is a close uniformit the slopes of the straight lines, in spite of the enormous differences of morp logical form and of time taken to accomplish the changes depicted. Thus chick series is accomplished in three weeks, the development of the squid



. 296. Creatine in the chick embryo (Mellanby) and in the rat (post-natal life; Chanutin).

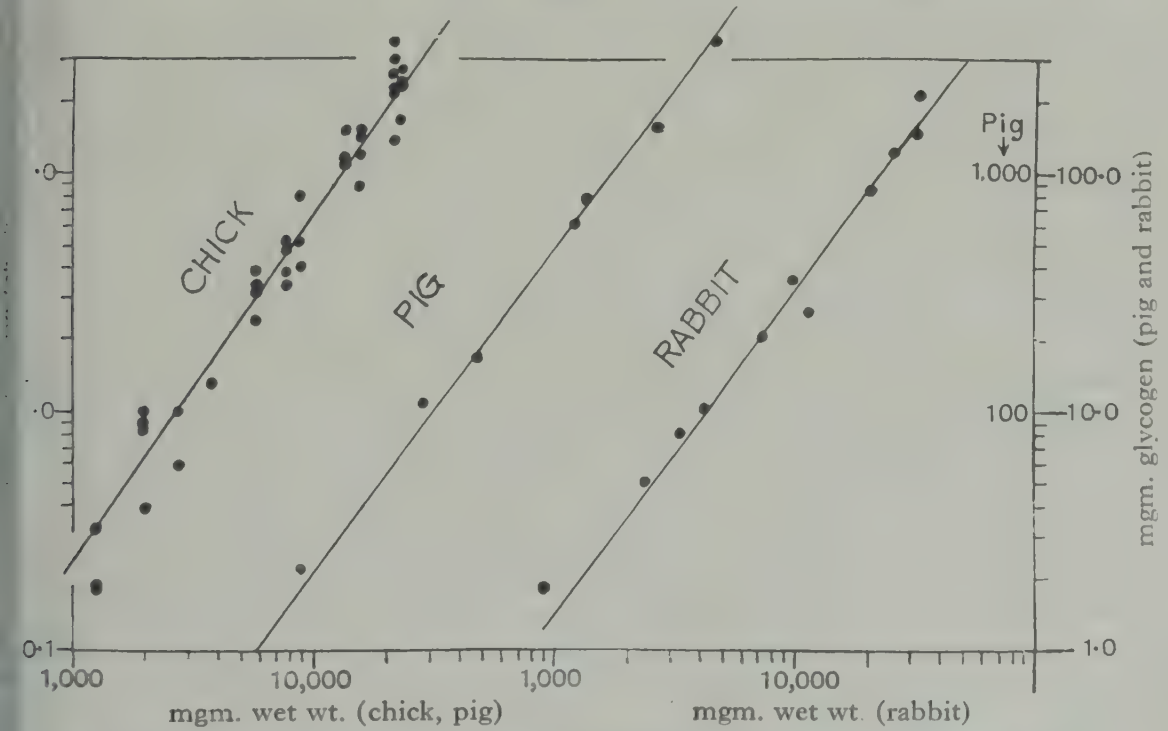


Fig. 297. Glycogen in the chick embryo (Vladimirov & Danilina); in the pig embryo (Mendel & Leavenworth) and in the rabbit embryo (Lochhead & Cramer).

the selachian fishes may take several months, and that of the rat more than a y
Again, the absolute values vary from a few milligrams in the mealworm
kilos in the selachian fishes. Finally, the nutritive factors are quite differ
The chick finds all the ash which it requires for its body in the egg interior
the shell, but the embryo of the squid has to absorb 30 % of the inorganic s
stances required from the sea-water surrounding its egg. As for the selach
fishes, *Scyllium* develops in a shell but nevertheless absorbs 75 % of its ash fr
the sea-water, while the others, developing ovoviviparously within the mate
body (but without placentoid attachments) absorb 25, 60 and 97 % respecti
of their ash from the maternal blood stream. The rat and the mealworm rec

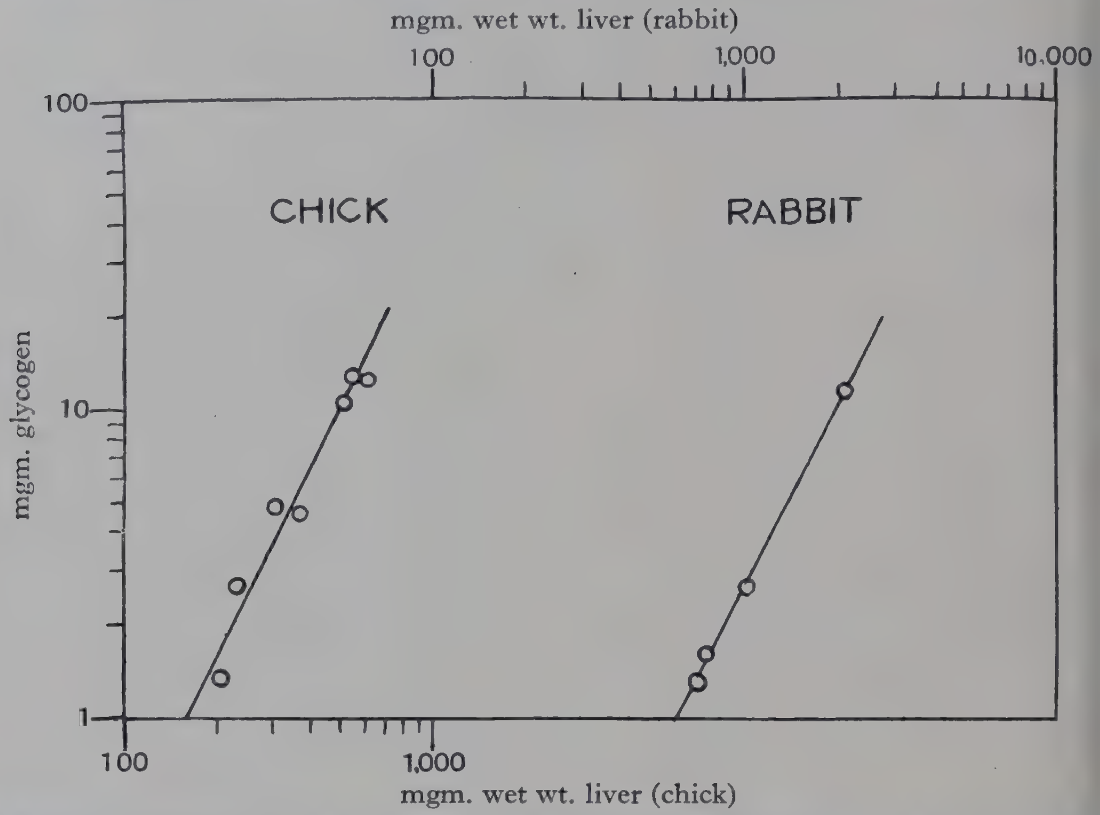


Fig. 298. Glycogen in the liver of the chick embryo (Vladimirov) and of the rabbit embr
(Lochhead & Cramer).

the ash in their food in the ordinary way. But in spite of these far-reach
nutritive differences, the heterauxesis is uniformly negative, as Table 30 dem
strates.

Among the constituents of the ash, calcium and phosphorus may be selec
for examination. Fig. 300 shows the calcium in the human embryo (Schmi
the chick embryo (Mankin[3]; Romanov[2]; and Plimmer & Lowndes) and the
(Sherman & McLeod). In all three series of data a closely similar slope
observable, showing that with increasing size the body contains relatively lar
quantities of the metal. Other ions, such as sodium and potassium, give pl
to it, just as chloride gives place to bicarbonate (see p. 585).* Thus from c

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* From this it follows that the K/Ca ratio of the tissues will be changing throughout embry
life, as was experimentally found by Kaufman & Laskowski. It is interesting in this connec
that according to Lasnitzki[1] the respiratory and glycolytic systems of embryonic tissues are m
less susceptible to serious deviations of K/Ca balance than adult ones.

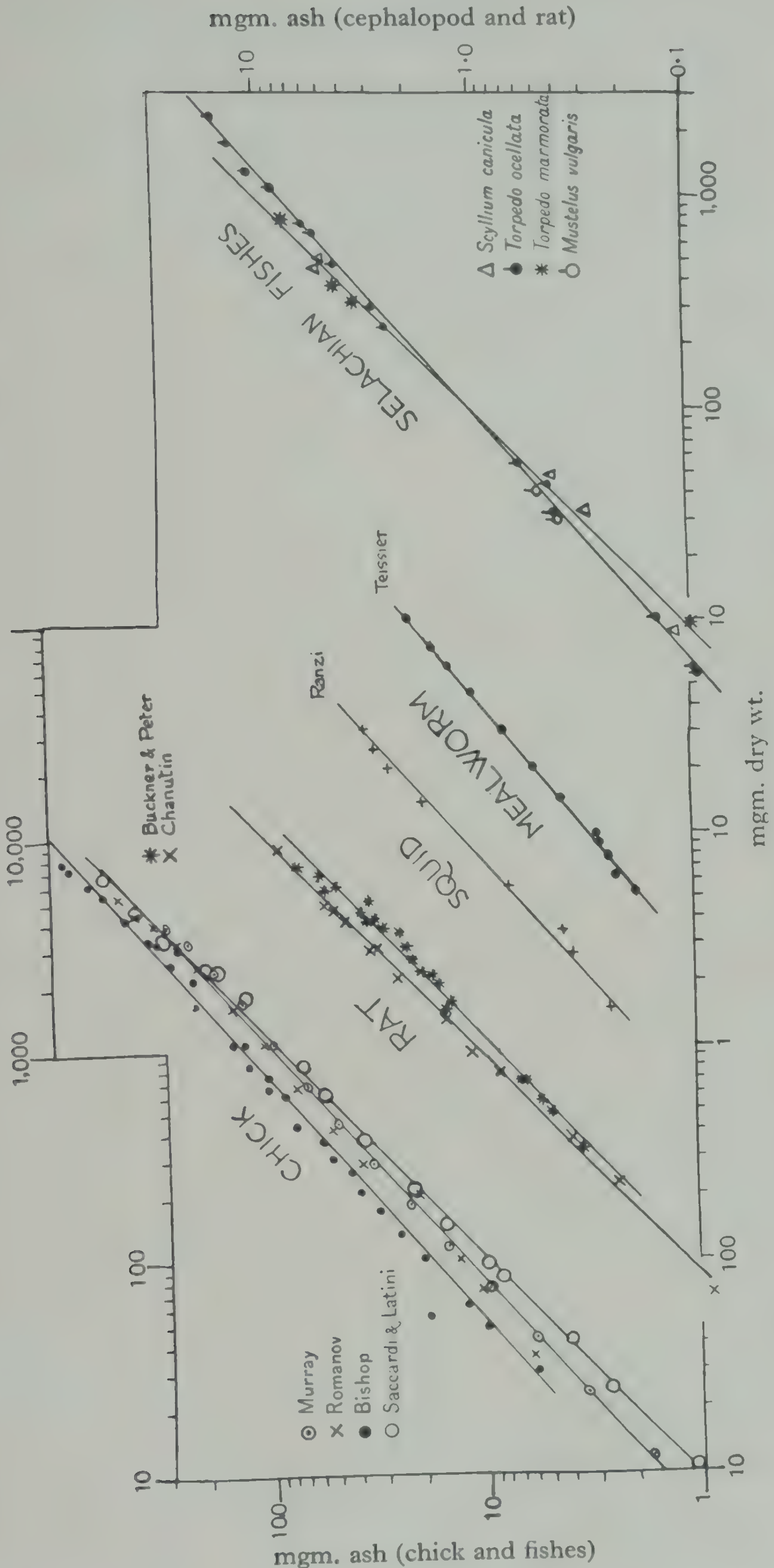


Fig. 299. Total ash in the chick embryo (Murray; Romanov; Bishop; Saccardi & Latini); the rat (post-natal life; Chanutin; Buckner & Peter); the squid embryo (Ranzi); the mealworm (larval life; Teissier) and various selachian fish embryos (Ranzi).

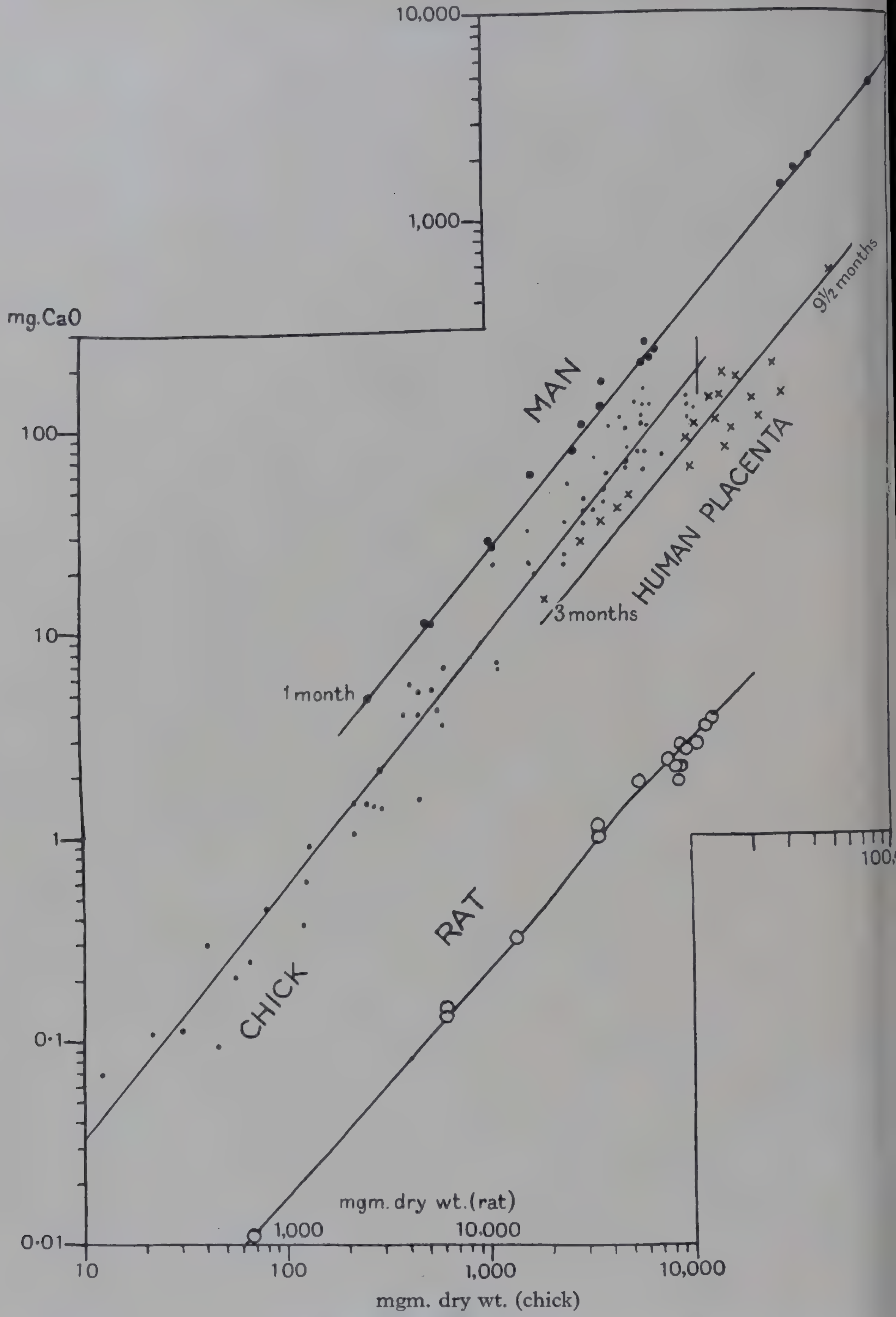
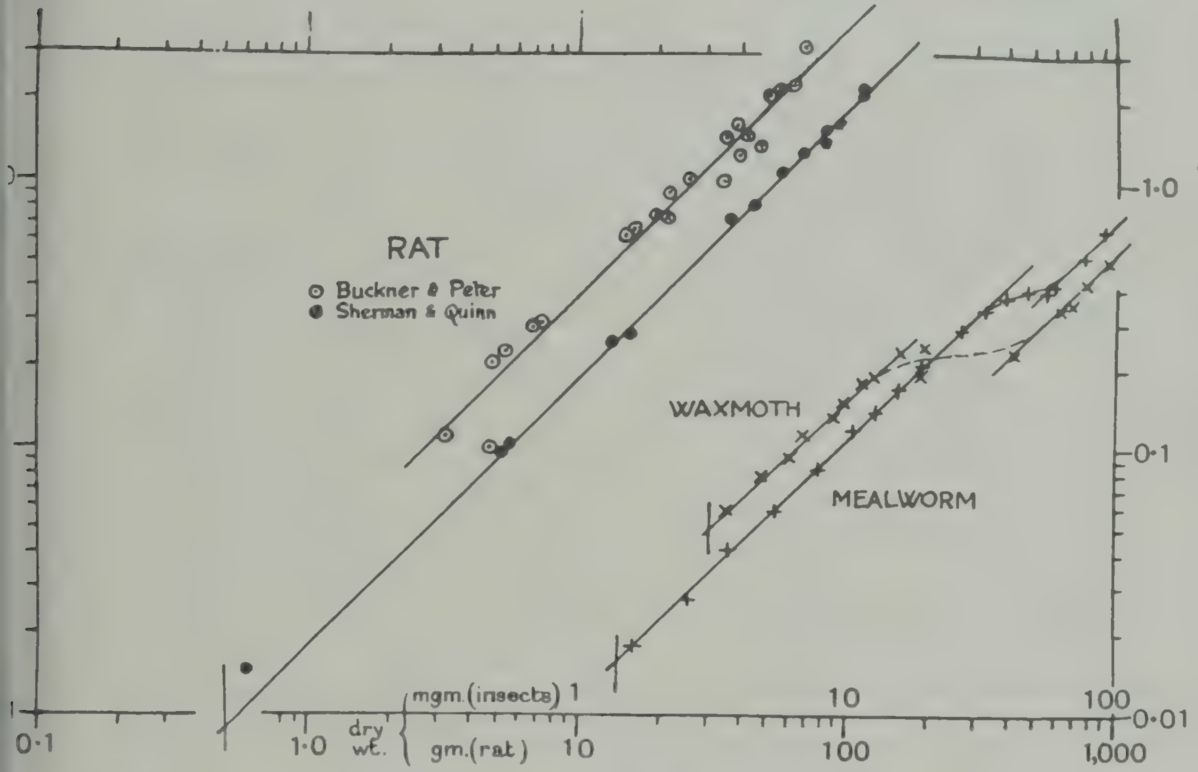


Fig. 300. Calcium in the chick embryo (Mankin; Plimmer & Lowndes; Romanov); in the hu embryo (Schmitz) and in the rat (post-natal life; Sherman & McLeod).

10·0 gm. dry wt. in the case of the chick embryo,* and from 0·2 to 100·0 gm. wt. in the case of the human embryo, the absolute increase rate of calcium he same. The data of Wehefritz for the calcium in the human placenta have n included on the same graph, but the points are too scattered and too limited locality to make the drawing of a straight line through them justifiable. It can seen, however, that a line drawn parallel to the embryo line does not greatly srepresent them, and it is quite possible that the growth of the placenta follows imilar course to that of other cells of embryonic origin. Here is the requisite ception for a new analysis of the constitution of the placenta.



301. Total phosphorus in the rat (post-natal life; Buckner & Peter; Sherman & Quinn); in the mealworm and in the waxmoth (larval life; Teissier).

he period of life covered in Fig. 300 is that during which ossification (see 55) is proceeding,† and at first sight it may appear strange that the onset of rocess is not accompanied by a change in the differential growth ratio. The clearly indicate, however, that the deposition of calcium in the bones is ly a matter of translocation of the element, and does not involve any relatively ter increase of intake at the same time.

nlike calcium, phosphorus is an element which shows an isauxetic relation. data for the post-natal life of the rat (Buckner & Peter; Sherman & Quinn) for the larval life of the mealworm and waxmoth (Teissier[1]), shown in Fig. 301,

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at much of the calcium of the embryonic body is derived from the egg-shell is a well-lished fact (*CE*, p. 1265). Glaser & Piehler have actually been able to demonstrate, by ng, the erosion grooves made by the allantoic blood-vessels on the egg-shell as the calcium bonate dissolves in the blood.

machi[1] tried to alter the rate of deposition of calcium and phosphorus in the chick embryo jecting various substances, but with little success.

illustrate this clearly. If any heterauxesis is present at all, it is very slig negative. The insect data here show changes in the constant *b*, as referre above. A biochemical interpretation of this behaviour is difficult, especiall total phosphorus includes so many separate fractions (lipin P and nuclein P l of which are bradyauxetic; and inorganic P, which is tachyauxetic, e.g. $k =$ for the chick (Baldwin & Needham[1]). The interest lies in the close similarit the mammal and the insects.

So far (except for liver glycogen) we have been discussing the changes oc ring in the body of the organism as a whole. It is interesting to examine development of individual organs and tissues, and of these a good instance the present purpose is the mammalian brain.* Donaldson & Hatai and Koc Koch studied the water content of the developing brain of the rat, w McArthur & Doisy investigated that of man, and Bäcklin that of the rabbit. Fig. 302 the dry solid in the brain of rat and man is plotted against the wet wei The correspondence between the slopes of the straight lines resulting is rem ably good ($k = 1{\cdot}38$ for the rat, $1{\cdot}33$ for man).

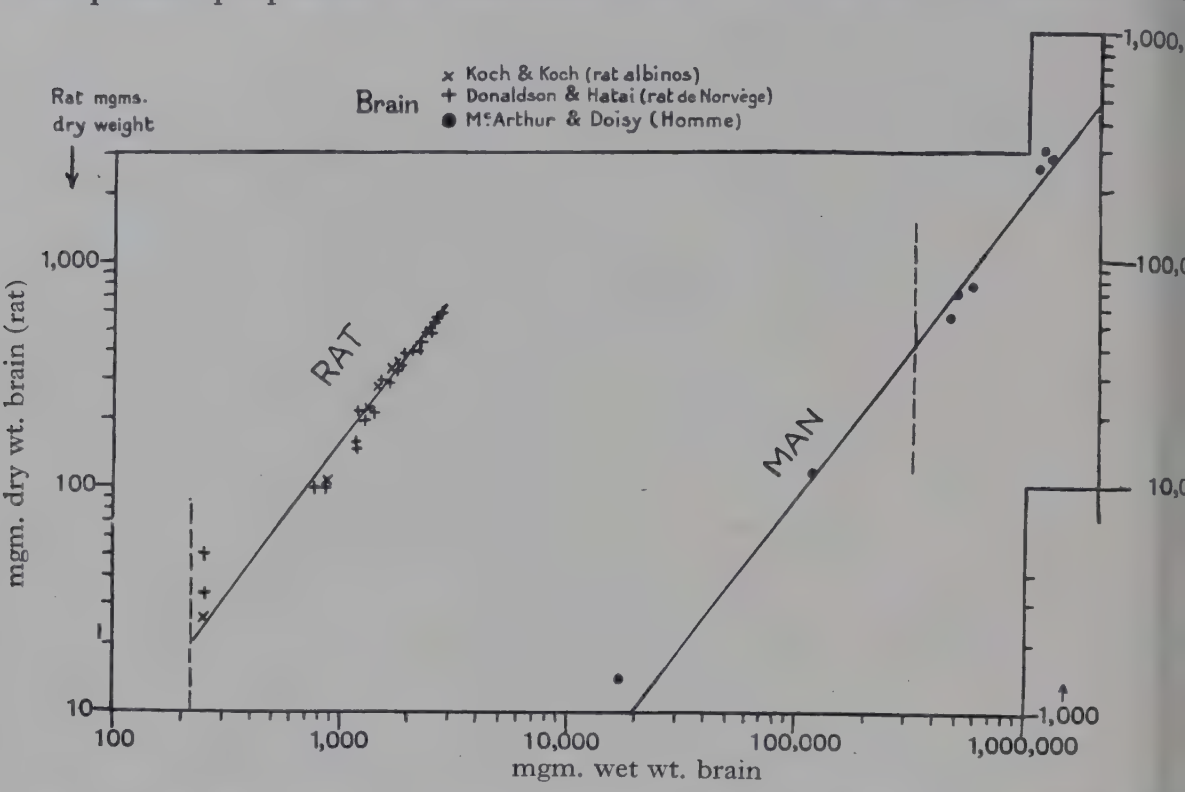

Fig. 302. Dehydration of the brain of the rat (post-natal life; Koch & Koch; Donaldson & H and man (pre- and post-natal life; McArthur & Doisy).

The correspondence between the general pictures of constituents for mammalian brains is, however, equally noticeable. Fig. 303 gives the data proteins, phosphatides, cerebrosides, sulphatides, organic and inorganic tractives, and cholesterol. It can be seen at a glance that although the gen level of absolute weight is quite different, and the time of occurrence of birt also different, the principal course of events is closely similar. The slopes ag

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* It is of interest that as long ago as 1898 Lapicque compared the ether extracts of brai different sizes, taken from a series of mammals. The available technique, however, was too c to permit of any sure conclusions.

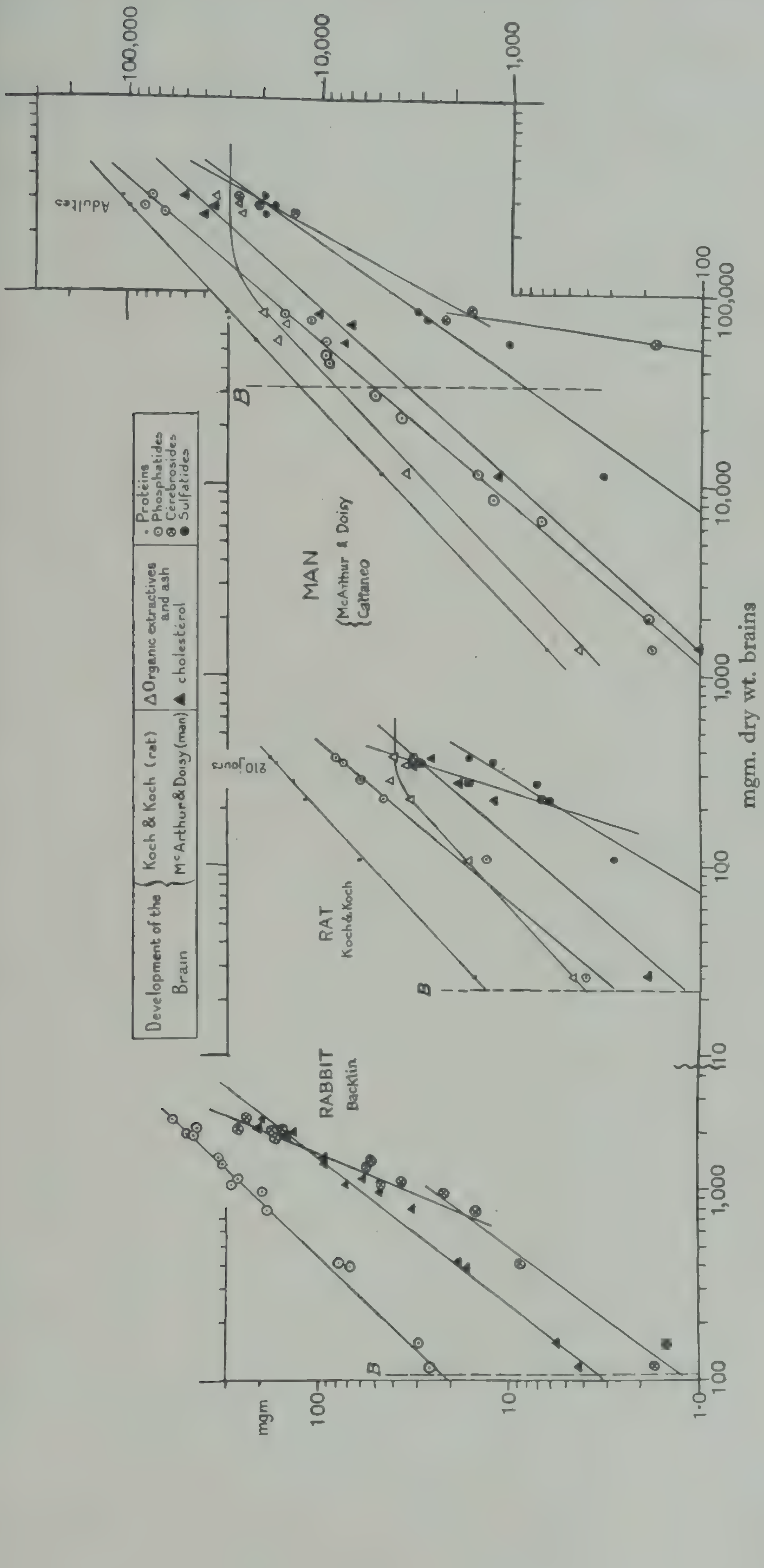

Fig. 303. Growth of the constituents of the brain of the rat (Koch & Koch); the rabbit (Bäcklin) and man (McArthur & Doisy; Cattaneo).

and each constituent increases relatively at a characteristic pace. The incr continues so long as the brain is growing except in the case of the org extractives and ash, a fraction which seems to come to completion at a cert point, and thereafter to increase no more. Particularly interesting is the v rapid increase of the cerebrosides, where sometimes two phases seem to indicated; it occurs last of all and is certainly associated with myelinisation. T numerical values and ranges of k are shown in Table 31.

Table 31. *Heterauxesis of the constituents of the mammalian brain*

Fig.	Constituent	k Rat	k Man	k Rabbit	Range of k	Aver k
302	Related to wet weight of brain:					
	Dry substance	1·38	1·33	—	0·05	1·3
303	Related to dry weight of brain:					
	Protein	0·94	0·94	—	0·0	0·9
	Phosphatides	1·21	1·17	1·03	0·18	1·1
	Cerebrosides (first phase)	3·11	7·11	1·35	—	—
	(second phase)		1·86	2·35	—	—
	Sulphatides	1·66	1·43	—	0·23	1·5
	Organic extractives and ash (until the point of completion)	0·92	0·95	—	0·03	0·9
	Cholesterol	1·15	1·12	1·28	0·16	1·1

From Table 31 we find that the proteins and the organic and inorgan extractives show definite bradyauxesis, while the phosphatides, cerebrosides ar sulphatides, together with the sterols, show pronounced tachyauxesis. This equivalent to saying that the structure is first laid down on a protein (one mig almost say water-soluble) basis, and it is only later on that the lipin and ster molecules come to take up their places in the nervous system. This is just wh we see in the chick embryo, for the histochemical work of Marza[1] indicates th in the nervous system, at the primitive streak and somite stage, there is very litt lipoid. It is as if the proteins represented the steel framework, and the lipins t concrete walls, of a modern building. There is, moreover, a certain reversibili in this succession, for R. M. May[1] has shown that during the course of degenerati of nervous tissue (whether produced by traumatic encephalitis or by section the sciatic nerve) there is an increase in percentage of water and nitrogen ar a decrease of lipoid. It is almost as if the chemical differentiation were retraci its course, but no doubt the phenomena of degeneration in nervous tissue a too complicated to admit of so simple a description. Nevertheless, the hete auxesis of degrowth remains an important problem, and it would be of gre interest to determine whether, in an organism such as a planarian worm, whe degrowth can be observed on an imposing scale, the chemical heterauxesis strictly reversible. There are already grounds for supposing that it would found to be so (see p. 525).

Another interesting possibility is that some forms of mental abnormalit especially the idiocy states, are due to failures in the normal chemical growt plan of the nervous system. The estimations of Ashby & Glynn lend plausibili to this suggestion.

Returning to the question of applying heterauxetic methods within the limits f one organ, the picture for the mammalian brain could be further filled in, if esired, by calculations from the data on lipins and sterols between birth and iaturity collected by Lang on the rat, and Schuwirth on man,* and the parallel ata on ash collected by Leulier & Bernard; Landa-Glaz; Goldenberg, Landa-ilaz, Voinar & Ostrumova. For the liver there are analyses on the pigeon by .aufman & Nowotna (though the number of separate points would be rather few), ıd we have already seen something of liver heterauxesis while discussing glycogen :cumulation. For muscle there are a few figures collected by Asmolov. Finally, Ieunier[2] has analysed the existing data for the chemical heterauxesis of developing ɔne, especially those of Hammett[5]. Table 32 gives the results for the femur of ıe rat, where there is a critical point at 150 mgm. (wet wt.):

Table 32. *Heterauxesis of the constituents of mammalian bone*

	k	
	Before the critical point	After the critical point
Organic substance	1·13	1·13
Total ash	1·12	1·80
Calcium	1·18	1·81
Total P	1·15	1·80
Mg''	—	1·56
Water	0·94	0·46

hus as ossification proceeds lime and phosphorus displace water, but the milder chyauxesis of the organic substance persists (see also Burns & Henderson). arbonate may also tend slightly to take the place of phosphate (Yoshida).

The actual mechanism of the deposition of ash in the bones of the embryo has :en the subject of a good deal of work. Robison's phosphatase[1] increases in tivity in calcifying bone whether *in vivo* or *in vitro*† (Fell & Robison[1]), though ıce the ash is continually being deposited, it declines on the dry weight after sing through a peak.‡ Similarly, prospective non-ossifying cartilage produces phosphatase *in vitro* while prospective membrane bone does (Fell & Robison[2]). :eoblasts are therefore correlated with an enzyme which makes inorganic ɔsphate from hexosephosphates, glycerophosphates, etc. and so brings about supersaturation of the tissue fluid with bone salt. But this is not the only tor; some other mechanism is concerned with promoting deposition from this dium (Robison, McLeod & Rosenheim). The two factors are difficult to ·arate (Shipley, Kramer & Howland; Robison[2]), but the latter, unlike the mer, is sensitive to cyanide, iodoacetate and fluoride (Robison & Rosenheim),

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Iere there is also older work by Fraenkel & Dimitz; Mansfeld & Liptak; and Bergamini, which y not be without value.

Ihere is an interesting connection here between the appearance of phosphatase and self-ferentiation. Third day chick embryo femora *in vitro* never develop epiphyses, etc., nor do they m phosphatase; fifth day femora do both. That the formation of bone shape is not primarily ditioned by muscle tractions or other *in vivo* conditions is now clear (Fell & Robison[2] on femora; l & Canti on knee-joints; Glasstone on teeth; cf. the monograph of P. D. F. Murray[3]).

t also does this when related to total nitrogen (Roche & Leandri), though the peak comes ier.

which suggests that the deposition of the ash may require energy derivable fr carbohydrate breakdown. Hence the glycogen in growing bone (H. A. Harr and teeth (Glock) may be of much importance. The calcifying mechanisms app in development before any visible calcification (Niven & Robison; Fell Robison[3]). When calcification is complete, excess of calcium or phosphorus in maternal diet produces no hypercalcification (Booher & Hansmann).

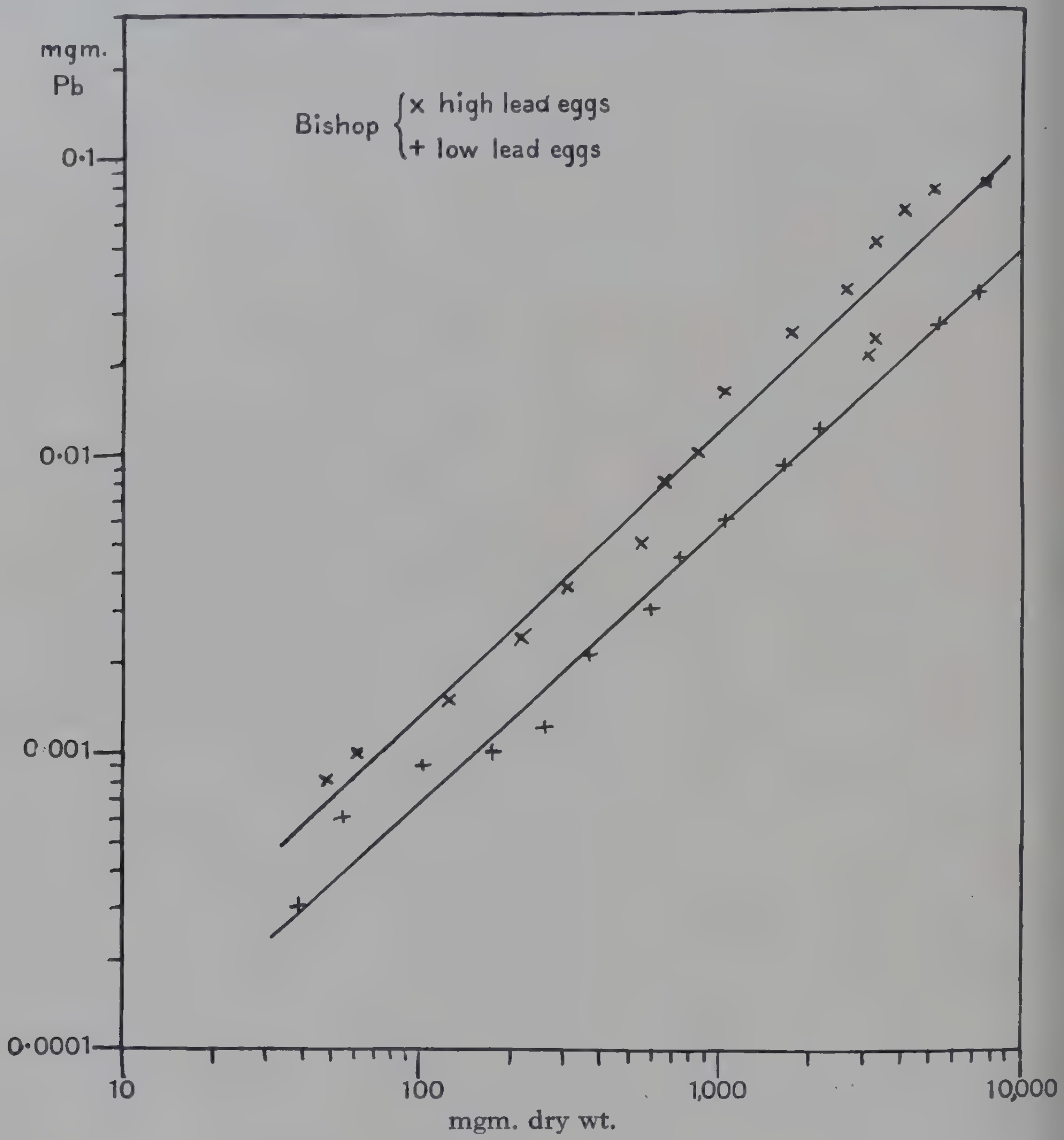
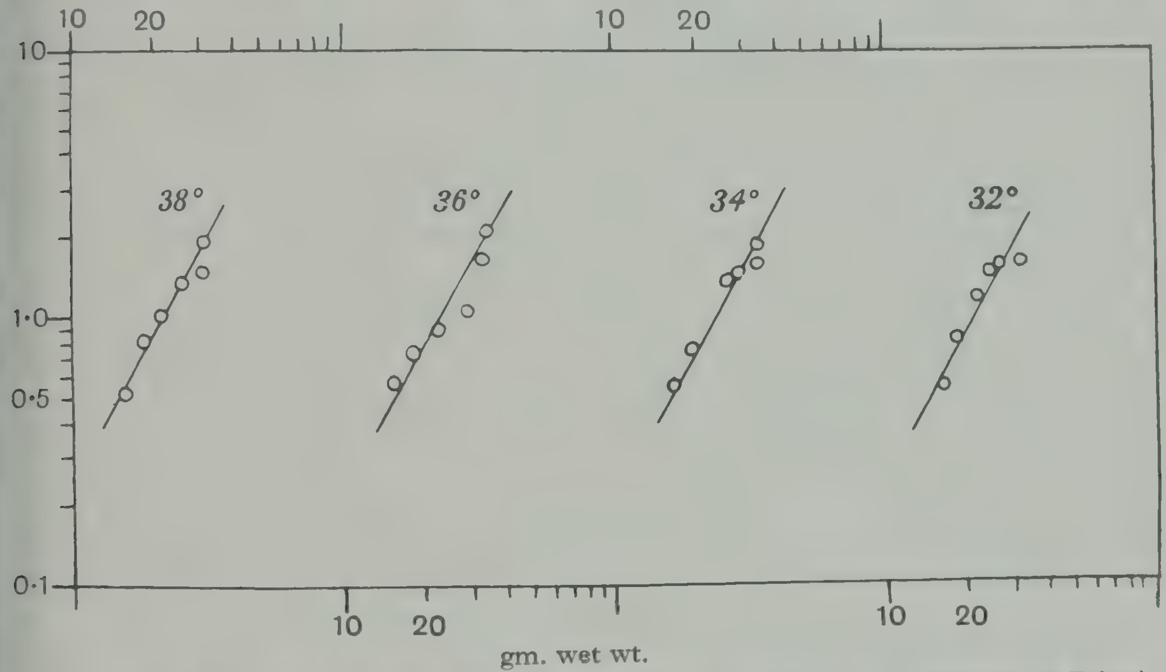


Fig. 304. Lead in the chick embryo (Bishop).

Little is known about the intrinsic nature of calcification. But Cagliotti Gigante have pointed out that the molecular spacing of hydroxyapatite is th same as that of the peptide chain, and Schour & Hoffman have discovered a bas calcification rhythm at 16μ intervals in teeth, possibly connected with calc spherite formation.

It was remarked in connection with the heterauxesis of total ash (Fig. 299) th the process of chemical differentiation seemed to be independent of the nutriti conditions of the animal. This phenomenon is found again in Fig. 304, whic gives Bishop's data[1] for the lead in the chick embryo. The entry of lead into th ick follows the same course no matter what the level of lead in the unincubated g, fixed by the diet of the laying hen, may be.[*]

Does temperature affect the heterauxesis of chemical substances? Can the mposition of the body at a given moment be varied by changes of external mperature, i.e. by speeding up or slowing down, the rate at which the whole owth process takes place? At present there are insufficient data in the literature give a satisfactory answer to this question, but a beginning has been made by omanov & Faber, who have studied the fat content, calcium content, etc., of ick embryos reared at different temperatures from 32° to 40° C. Their results r the former group of substances are shown in Fig. 305, where fat is plotted ainst *wet* weight for four different temperatures. The experiments, as far as ey go, indicate a uniform k (1·79 at 38°, 1·77 at 36°, 1·85 at 34°, 1·84 at 32°). t they are unsatisfactory; first, because only a very small range of growth was en (15–35 gm. wet wt.), and secondly, because in the chick embryo large iations of temperature are not possible without the death or damage of the bryo. Work of this kind should be done on amphibian embryos, where the nperature may vary from 8 to 21° and the time of development from 30 to lays. A similar experiment to that of Romanov & Faber was carried out by rner & Gunns on the leg-bone lengths of the chick embryo incubated at ferent temperatures; k was not appreciably different. It may be foreseen that nperature, and hence the speed of the whole process, will have little or no fect on chemical heterauxesis, for it is now fairly clear that at least the efficiency ne material stored in relation to that combusted) is, within ranges of tempera- res permitting normal development, nearly always the same (see on this the scussion in *CE*, pp. 937 and 973). The question should, however, be experi- ntally tested.



ig. 305. Fat in the chick embryo incubated at different temperatures (Romanov & Faber).

The work of Melampy, Willis & McGregor indicates that small differences in chemical heter- esis may underlie the divisions into "castes" found in the social insects (cf. p. 309).

3·25. The chemical ground-plan of animal growth

Let us now consider what is the general significance of the work reviewed this account. When we plot the magnitude of a chemical constituent of organism against the magnitude of the organism as a whole, we have to do w a rather high level of abstraction.

(1) We are abstracting from the morphological form.

(2) We are abstracting from the factors of nutrition.

(3) We are abstracting from the absolute values of the magnitudes. T relation of chemical entity to chemical totality may run an identical course organisms of widely different size.

(4) We are abstracting from the time factor. At whatever speed the proc of growth takes place, or whatever its position on an absolute time scale, t relation of chemical entity to chemical totality will be manifested as the same it *is* the same.

What, then, is left? Nothing but a system of ratios or relations, which m possibly be the same in all embryos, and which certainly seems to be the same many embryos, in a word, a chemical ground-plan of embryonic growth. T disturbing influence of time makes this plan difficult to see when growth considered as a function of time, but in heterauxetic plotting, the time factor short-circuited, i.e. made implicit, and the plan revealed.

It may be well to enlarge upon the processes of abstraction listed above. T fact that organisms of extremely different morphological form give identic differential growth ratios for a given chemical substance might at first sight taken to mean that genetic differences and interphyletic differences occur on at a supra-chemical level. Not "protoplasm" only, but also the changes whi it undergoes in chemical constitution during the growth of the organism, wou then be identical in all animals, and the genes would produce different rabbi out of the same hat, just as the sculptor produces a variety of forms out of homogeneous substance. But it must be remembered that chemical heterauxesi as so far discussed, refers to two separate levels of analysis. Out of the sevente substances which have been discussed as chemical entities in the precedi account, seven are chemically well-defined (e.g. water, glycogen, lead) and t are more properly speaking groups of substances (e.g. non-protein nitrogen, as sulphatides). The problem of how far every well-defined chemical substance the body will be amenable to heterauxetic treatment must await further wor Meanwhile, it is clear that even in the extreme case where every given group substances had the same heterauxesis in different animals, there would still a great deal of scope for genetic differences at the chemical level. "Sulphatide for example, might show a *k* identical for supra-oesophageal ganglion and f brain, but its chemical structure (position and nature of side-chains, etc.) mig vary considerably as between crustacea and mammals. One has only to think the specificity of animal pigments or of immunologically active proteins to s that whatever else uniform heterauxetic relationships mean, they cannot po sibly mean that "protoplasm" is the same everywhere. The classical work ichert & Brown on haemoglobins, and of Reichert on starches, together with e early studies on the racemisation of proteins (Dakin & Dudley; Dudley & oodman) established long ago that profound interspecific differences exist in e molecular structure of different proteins. If "protein", therefore, gives us identical k for different animals, it is much more likely to be due to some iestion of colloidal stability, as suggested above.

This brings up the question of how far senescence (a process obviously inclusive that of growth) affects chemical heterauxesis. For the present, although an sociation between senescence and colloidal stability is guessed at (cf. Dhar), cannot suppose that age as such has much to do with chemical heterauxesis. ter the cessation of growth, there is little change in chemical composition until e end of life, as is well shown in Fig. 306, modified from Moulton's well-known per. On this subject there is a review by McCay.

In this connection the question may be asked whether if growth were greatly tarded or accelerated heterauxesis would follow suit. When the growth of rats is severely retarded by restricting the calorie intake, heart size was larger and er size smaller than normal for the weight in question, though no biochemical ta were obtained (McCay, Crowell & Maynard). But within limits of growth-te more near the normal the water-content of tissues (muscle and thymus) ried solely with size and not with age (Moment) as also did the chemical mposition of the bones (Outhouse & Mendel) and their length (Lerner & unns). The same is true of the water-content of larval amphibia (Galloway); p. 524. Further investigations on the temporal deformability of heterauxetic ations would be of interest.*

The present treatment constitutes a step towards a theory of biochemical insformations. It cannot be too much emphasised that although the time factor short-circuited in heterauxetic graphs, it is not completely eliminated from the oblem. On the contrary the chemical heterauxesis of different animals should into one common plan if they were reduced to the same time scale and the ie absolute size. This has been shown by Waddington[4] in the following way. ve have two animals p and q, and measure in each of them several chemical gnitudes M_p, M_q, N_p, N_q, etc., we find relations of the type

$$\log \frac{M_p}{M_{p_0}} = k \log N_p \qquad \text{......(12)}$$

1

$$\log \frac{M_q}{M_{q_0}} = k \log N_q \qquad \text{......(13),}$$

---

he pituitary growth hormone should provide opportunity for testing whether the chemical position of the body depends on size or on age, but the data so far obtained are not quite ir. In hypophysectomised rats the bradyauxesis of water and the tachyauxesis of fat seen in mal growth cease, and the animal maintains approximately the same composition which it had en growth was arrested by the operation (Lee & Ayres). Thus composition depends on size and on age. But in rats gigantised by administration of extra growth-hormone the water loss and fat gain seem to be inhibited and the abnormally large animal has much the same composition t had at the beginning of the treatment (Lee & Schaffer), i.e. more water and less fat than normal control of the same age, though if composition depends on size and not on age it should e exactly the opposite, since in terms of weight it is "older". The question needs further lysis.

where M_{p_0} and M_{q_0} are specific constants, and k a general constant relating and N for all animals. Now M and N are also functions of the time t. If we ha

$$\log \frac{M_p}{M_{p_0}} = F(t) \qquad \ldots\ldots(14$$

and

$$\log \frac{M_q}{M_{q_0}} = F(t) \qquad \ldots\ldots(15),$$

we can choose another variable ϕ such that

$$F(\phi) = F(t) \qquad \ldots\ldots(16).$$

That is to say, by choosing a suitable unit or function for the measurement time, we can convert the growth curve of M_p into that of M_q; and further, t same system of time measurement will convert all the growth curves of chemic magnitudes of animal p into those of animal q, provided only that in each ca

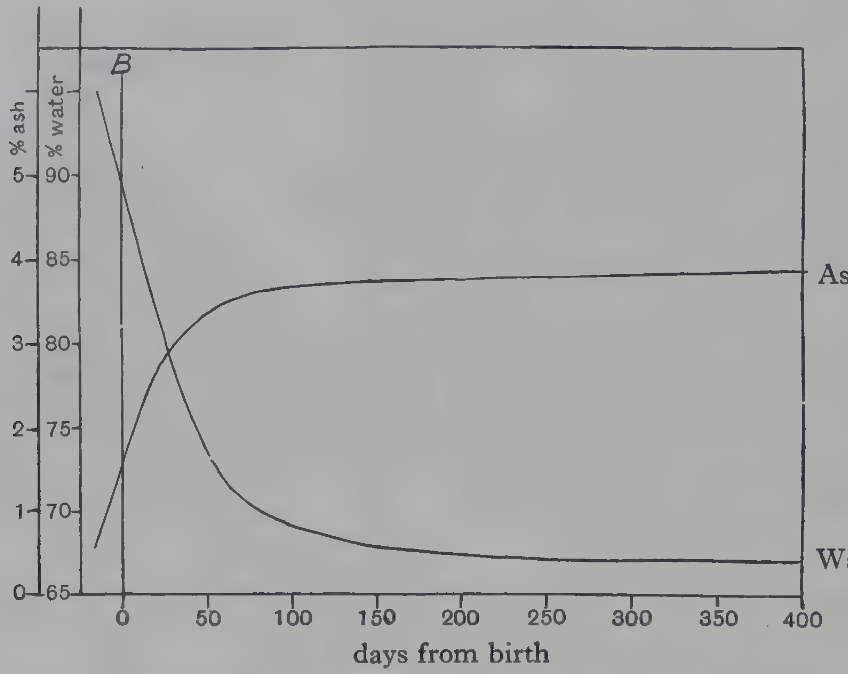


Fig. 306. Percentage of water and ash in the rat throughout the life cycle (modified from Moulto plot of the data of Hatai; Zuntz; Inaba; Chanutin; Buckner & Peter; Sinclair).

there is a linear relation between the logarithms, with the general constant Then we could regard the two systems of time measurement defined by t and as the relative time scales of chemical development in the two animals. B relative time scales may be derived in other ways, e.g. from morphologic development, and from embryonic determination. The morphological stage which an organ becomes embryologically determined may vary widely in relat species. It would be interesting to know how the time scales of morphologic determinative, and chemical development, are related to one another.

If, then, the unitary chemical ground-plan of animal growth exists, we mu think of it as deformable in space-time. Just as d'Arcy Thompson was able, systematic deformations of Cartesian co-ordinates, to transform one morph logical shape into another (e.g. the sun fishes, the amphipods, mammalian skul etc.), so the chemical ground-plan is deformed in time and space. It can be slow down or speeded up, and it can vary dimensionally from the extreme of t hyosaur to the extreme of the pocket gopher. But for the most part, if the ial magnitudes are reduced, the temporal magnitudes will be reduced too, it is here that we touch upon the thought of Lambert & Teissier who have posed as a fundamental biological law that homologies exist between animals ime as well as in space. There is an equality, they suggest, between the ratio omologous spatial magnitudes and the ratio of homologous times, and they port their "theory of biological similarity" by the existing empirical data for liac frequency, metabolic rate, gestation time, longevity, etc. At one point closely approach the present discussion, for they suggest that "at homologous ants (in the life cycle) two homologous organs will have the same qualitative quantitative (chemical) composition". Mouse time must bear the same, or milar, relation to elephant time as mouse spatial magnitudes to elephant ial magnitudes. Indeed, unless the time factor is brought into account, we understand morphological similarity, but we can never hope to understand siological, still less embryological, similarity. Analogous ideas have found ession in the short note by Carrel[2], the paper of Brody[1] and the books of Noüy and Lacape, who analyse the notion of "physiological time". Here, Hoagland's possibly radioactive "physiological clock", we tread upon the ders of psychology, but it may be that the sense of duration will some day ound to be not so far removed from embryonic events as we may at present gine.

ambert & Teissier, then, suggest that animals of the same general form, but ifferent sizes, have the same form in space-time. The investigation of chemical rauxesis leads to the suggestion that animals of different form and different have the same basic general chemical plan of growth, which is deformable in wide limits in space-time. The process of growth would thus proceed rding to a definite plan recognisable in the constitution of the organism at given stage of its life history. Potentiality offers to Actuality a formula in h substitution may be freely made from a wide, but not infinite, range of s.

regards the validity of the conclusions which can be drawn from similarities it is clear that the main difficulty lies in knowing how close values of k must order to permit a conviction of identity. This is essentially a statistical em. Unfortunately, from the ranges of k given in Tables 29, 30 and 31, impossible to argue far, for in each case k depends, at least partly, on the lard deviation, probable error, etc., of each of the individual sets of data on h it is founded, and the statistical qualities of the data are not often known. could they now be calculated, in view of the peculiar conditions attaching ch individual investigation and the particular chemical method employed. e the data are so heterogeneous it is surprising that we find as many regu- es as we do, and these can hardly be thought the result of chance or coin- nce. What is required is a new and extensive investigation, carried out with same chemical methods and sampling, on a selected number of widely rent organisms at all stages of the life cycle and, if possible, under constant, n, and various, environmental conditions. The present account probably as far as it is possible to go by simple analysis of the published information.

It seems improbable, however, that the correspondences already noticed illusory. In Fig. 299, for example, where the range of variation of k is ra wider than usual (0·14), it may be observed that the range for the chick em alone is over half the total range, i.e. 0·09. This may be interpreted as favour to the view that the constants would correspond more closely than they do i data were better.

The general upshot of this section may be summarised by saying that h auxetic plotting allows us to recognise similarities between the developm plans of different organisms which, owing to the great differences in their t scales, would otherwise remain hidden. We always find the following distribu of relative growth rates:

I. *Isauxetic constituents*
  (to dry weight): protein nitrogen, total phosphorus.

II. *Tachyauxetic constituents*
  (to total weight): dry substances.
  (to dry weight): fat, creatine, glycogen, calcium, possibly magnes inorganic phosphorus, bicarbonate.

III. *Bradyauxetic constituents*
  (to total weight): water.
  (to dry weight): non-protein nitrogen, purine nitrogen, glutathione, ash, sodium, potassium, chloride, lipin phosphorus, nuclein phosph iron, copper, lead.

In this way we arrive at a universal ground-plan of chemical developm It is safe to say that when all exceptional cases have been considered, and w all criticisms of the heterauxetic treatment have been taken into accoun nevertheless remains the most convenient way of comparing the chem development of one organism with that of another.

3·3. Respiration

Preceding pages have often emphasised the fundamental fact that, apart f certain exceptional cases, embryonic growth and morphogenesis cannot pro in the absence of oxygen. How exactly the oxido-reductive energy-provi machinery engages with the differentiation processes is still very obscure, must some day be understood; meanwhile a few hints may be rememb (p. 474 and p. 498).

3·31. Respiration of the eggs of Echinoderms, Worms and Moll

For over thirty years the alecithic eggs of echinoderms, in view of t uniformity, transparence, and many other convenient qualities, have be favourite material for studies on the physiology of cell respiration.* Wit g back beyond 1931 we have here to review the most significant recent work, hich there has been an abundance. Although we are still far from a real tion of the essential problem, namely, how the chemical transformations and gy changes proceeding during development are integrated with the morpho- tic changes, this section should be read in conjunction with that on deter- ation in echinoderm development (2·9).

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* The technical difficulties, not so much of work with the eggs of marine invertebrates, comparisons between the work of different investigators, should not, however, be minin The determination of the volume of the material by centrifugation and the weight by calcul from Kjeldahl analyses, long customary, needed the critique which it has received from G & Rubenstein; Rubenstein & Gerard[1]; Whitaker[8]; and Shapiro[2]. Shapiro[3] has provided a u nomogram for calculating the force of a given centrifuge in terms of gravities.

rhaps the most classical result of the earlier investigations was the rise in iratory rate of the sea-urchin egg on fertilisation (*CE*, p. 636). This pheno- on has been repeatedly confirmed (e.g. by Whitaker[6]; T'ang[1] and L. Chesley on *cia punctulata*; by Borei[1] and Laser & Rothschild on *Psammechinus miliaris*; by Brock, Druckrey & Herken on *Paracentrotus lividus* and *Sphaerechinus ularis*). It is true that the immediate rise, which may be well above 400 %, t always maintained, and the respiratory rate settles down to a slower level, gh still well above that of the unfertilised egg. But it was known that some ies, such as the starfish, showed no rise at all on fertilisation, and this also een frequently confirmed (e.g. by T'ang[2] on *Asterias glacialis*). When the of a wider range of invertebrates were studied, many different types of viour were found. Thus the eggs of the clam, *Cumingia tellinoides*, studied hitaker[4],* showed a decrease on fertilisation to about 45 % of the pre- lisation rate, and the same was found to hold good for those of the annelid *topterus* by Whitaker[6]; L. Chesley and J. Brachet[16]. For another annelid, *is*, the position is not quite clear, for while Barron[2] found absolutely no change spiratory rate at fertilisation, Whitaker[5] found an increase of about 140 %.

hitaker has essayed a general interpretation by showing that (apart from the rvations of T'ang[1] and T'ang & Gerard, which do not fit into his scheme) the ratory rates of the embryos shortly after fertilisation are roughly of the same r (1·3 to 2·0 c.mm. O_2/hr./10 c.mm. eggs at 21°), although the respiratory of the unfertilised eggs may be very different. There would thus be a ing at fertilisation in the mollusc and annelid examples, and a removal of tion in the case of the echinoids, while the asteroids would show no change. ps it is too early to decide whether this generalisation meets all the facts. er investigations are still required.

e respiratory quotient has often been calculated, but as there is much ion in the results obtained, it is likely that the technique of measurement is eficient. The general tendency of the earlier work (cf. *CE*, pp. 623, 989) d it at unity or a little below, and this was confirmed by the finding of 0·9 rei[1] on *Psammechinus miliaris*, and 0·92 by van Herk on *Sphaerechinus*. But ussi[4], working on *Paracentrotus lividus*, obtained a figure of approximately r the period between the two-cell stage and the hatching of the blastula, his did not vary between 10° and 25° C. On the other hand Laser & Roths- , using a modern and carefully thought out technique, obtained 0·84 for

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itaker[3] also determined the change of oxygen consumption in the "eggs" of the alga, *Fucus osus*, on which important studies of polarity have been made (see p. 668), and found that s an increase of 190 %. In a later paper, he reported the odd fact that though the Q_{O_2} of ggs falls on fertilisation and that of sea-urchin eggs rises, both show increased stainability rtain dyes such as methyl violet and gentian violet after fertilisation (Whitaker[17]).

0–30 min. after fertilisation (*Psammechinus miliaris*) but 0·66 for 5–20 r afterwards. A still later paper, by Öhman of Runnström's school, gives R.Q. of *Paracentrotus lividus* eggs as 0·73 in the earliest cleavage stages (1–2 after fert.) and 0·85 after 7–8 hr. (presumably blastula stages). If this is corr the sea-urchin embryo would join the amphibian embryo (see p. 588) in havin carbohydrate quotient at gastrulation, and the succession of energy-sources Section 3·37) would start only from that point onward. There is no doubt that measurement of the respiratory quotient of sea-urchin eggs is a rather diffi task, partly because of the considerable acid production which occurs sho after fertilisation (see p. 572).

The effect of temperature upon the respiratory mechanisms of the echinod egg was studied by Rubenstein & Gerard[2], who found that the temperat coefficients of respiration before and after fertilisation in the sea-urchin (*Arba* are different (Q_{10}, 4·1 before and 1·8 after). The increase of oxygen consumpt on fertilisation therefore varies markedly with temperature, being tenfold at and only double at 30°. By extrapolation, there would at 32° be no rise at These results were later confirmed by Korr[1], as will be mentioned later, thou the eggs of other invertebrates do not show the effect clearly (Tyler & Humaso Ephrussi[4] sought an answer to the question whether the total amount of oxy consumed is identical at all viable temperatures, and found (using *Paracentrot* that for the period from the two-cell stage to the hatching of the blastula th is between 10° and 23° no variation in this quantity. From fertilisation to two-cell stage, however, constancy was only found between 17° and 23°; bel these temperatures the oxygen consumption fell off to −40 % of the normal 10° and above them rose to +40 % at 24·3°. The substantial constancy sho throughout development in these results was later confirmed by Tyler[5], as be mentioned in another connection (p. 574).

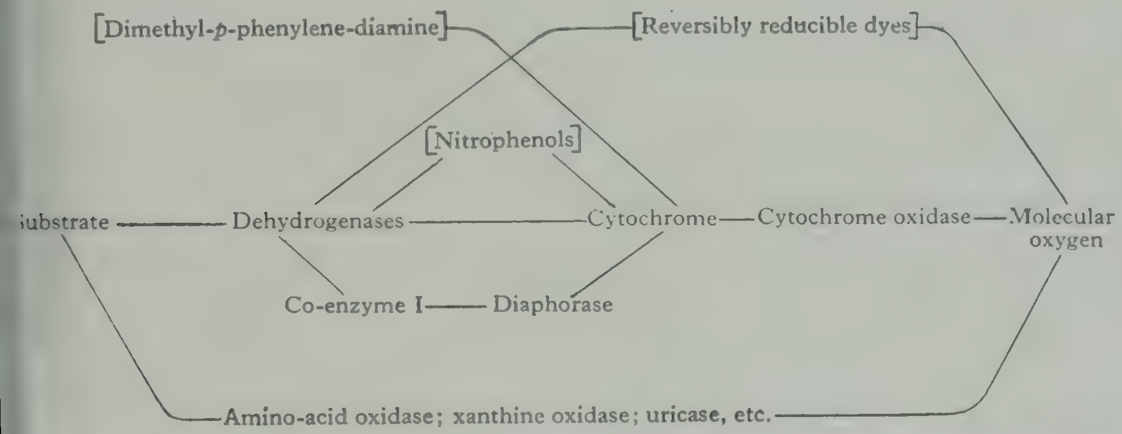
Ephrussi[4] also calculated the Q_{10} at different temperatures for respiratory ra and found that, contrary to the original Loeb-Wasteneys view, it came very cl to the Q_{10} for velocity of segmentation. This conclusion, that developmental r and respiratory rate cannot be dissociated by temperature, was again confirm by Tyler[5].

The relation between oxygen tension and oxygen consumption by echinode eggs was studied on unfertilised *Arbacia* eggs by T'ang[3] and on fertilised *Arba* embryos by Amberson and T'ang & Gerard. There is general agreement t respiration continues at 100 % of its normal level from 160 mm. Hg press (air) down to about 40 mm. Hg, after which point it rapidly drops, reaching z when the oxygen pressure becomes negligible. T'ang has reviewed this effect comparison with data on other respiring systems, and it has received math matical analysis at the hands of Gerard[2]; Rashevsky[2], and Landahl.

Anaerobiosis of course stops echinoderm development completely (E. Harvey[1]), but in some forms, such as the polychaete worm *Nereis* (Barron fertilisation is still possible under anaerobic conditions. Permeability of t *Arbacia* egg to water is slightly but significantly decreased in the absence oxygen, according to Kekwick & Harvey, but there is no difference in t equilibrium values attained. Hunter thinks there is no effect at all.

The effect of pH, CO$_2$, etc. on echinoderm egg respiration has also been the ject of some study. As might be expected, respiration falls off on both sides he normal external pH (Ashbel[2] on *Paracentrotus* and *Arbacia*), and the same rue of osmotic pressure (Borei[2] on *Asterias glacialis*). Excess CO$_2$ in the sea-er retards cleavage (Haywood & Root[1] on *Arbacia*); the effect is perceptible ensions as low as 4 mm. Hg and progressively increases up to 125 mm. Hg, which point segmentation is suppressed. Respiration of *Arbacia* eggs is inished by all acids, but by CO$_2$ more strongly than HCl at the same pH on ount of its greater penetrating power (Root). At a given CO$_2$ tension the sence of bicarbonate in the sea-water lessens the cleavage retardation men-ned above proportionally to its concentration, according to Haywood & Root[2]. this connection it is interesting that carbonic anhydrase, the enzyme which lyses the decomposition of bicarbonate, was found by Brinkman to be sent in echinoderm eggs.

cid pH may have curious effects on fertilisation. Thus in the gephyrean m *Urechis* Tyler & Schultz found that fertilisation can be blocked by sea-er at pH 7·2. If eggs are placed in this medium as soon as 3 min. after mination, they will show no development, and this block is reversible up to min. but later irreversible. Nevertheless these eggs retain their fertilisa-y, and upon transfer to ordinary sea-water and re-insemination, polyspermic elopment will ensue. This was subsequently generalised to the eggs of many ertebrate forms by Tyler & Scheer.


[Dimethyl-p-phenylene-diamine]
[Reversibly reducible dyes]
[Nitrophenols]
ubstrate
Dehydrogenases
Cytochrome
Cytochrome oxidase
Molecular oxygen
Co-enzyme I
Diaphorase
Amino-acid oxidase; xanthine oxidase; uricase, etc.

07. Scheme of cell respiration. Added agents not occurring naturally are placed in square ckets. Between the dehydrogenases and cytochrome or flavoprotein there intervene the acid cycles of Szent-Györgyi and Krebs (see Elliott's review, and here, pp. 597, 603,).

this point it is necessary for the understanding of what follows to refer to eme of cellular respiration summarising our present knowledge (Fig. 307). It built partly upon facts derived from the study of echinoderm eggs, and all was by no means clear at the time when some of the most important riments on them were made. As is generally realised, the major part of the iration of animal cells goes through the dehydrogenase and Warburg-Keilin ms (the central line of the diagram). Hydrogen of the substrate, activated by dehydrogenases, reacts with the reversibly oxidising-reducing substance hrome, and this in turn reacts with molecular oxygen activated by the en- cytochrome oxidase (formerly called "indophenol oxidase" or the *Atmungs-*

ferment). Since the Warburg-Keilin system is composed of iron-contai tetrapyrrol substances, respiration is generally inhibitable by KCN and CO. ' it is not always so inhibitable, however, shows the existence of alternative tr represented in the diagram by coenzyme I (see p. 209) and diaphorase (simil the yeast flavoprotein formerly called the "yellow enzyme" of Warburg[4]), th these may sometimes go through cytochrome; and mechanisms such as amino-acid oxidase, xanthine oxidase, etc. which undoubtedly react direct oxygen.

At the same time a number of substances are known which have the prop of greatly increasing the respiratory rate of certain cells. The reversibly oxidisa reducible dyestuffs are the best known of these; they act by side-tracking Warburg-Keilin system, as we know from the fact that the respiration catal by them is unaffected by KCN and CO, the inhibitors of "ferrous" respirat Then the substance dimethyl-*p*-phenylene-diamine, known from a much ea date than the dyes, behaves in a different way. With α-naphthol, as the clas Rohmann-Spitzer reagent, much used in histochemical work (cf. p. 136) oxidative condensation occurs forming the dye indophenol blue, but even witl α-naphthol there is a condensation giving a coloured product (Keilin[2]). It is clear (Keilin & Hartree) that dimethyl-*p*-phenylene-diamine reacts with c chrome and not directly with the "indophenol oxidase", so that its action be said to be analogous to introducing a large quantity of fully activated subst into the system. Thus the dehydrogenases and normal substrates are side-track Last come the compounds of nitrophenol type. We know that these are autoxidisable; they must act through the Warburg-Keilin system, since excess respiration catalysed by them is fully inhibitable with KCN and (Krahl & Clowes[1]; de Meio & Barron); and they do not affect the nature of substrates used since they leave the respiratory quotient of the cells unchan (Krahl & Clowes[1]). What they do is to accelerate the reduction of cytochrome the oxidation of its substrates (Krahl & Clowes[2]); a stage which must in many therefore be normally a limiting factor.

The nitrophenols also accelerate fermentation, but it is likely that their act there is of a different kind (Ronzoni & Ehrenfest). Nevertheless it is interest that Krahl & Clowes[2] showed that the greatest stimulations of fermentat occur with those yeasts having normally the lowest fermentation rate and *versa*.

The foundations of our knowledge of respiratory mechanisms in echinod eggs were laid by Runnström[7]. Working on the eggs of the sea-urchin *Paracentr lividus*, where the post-fertilisation respiration rate is four or five times that bef fertilisation, he established that KCN strongly inhibits the respiration of fertilised eggs* but has no effect on the unfertilised ones, and that CO stron inhibits the respiration of the fertilised eggs but has a definite acceleratory ef on the unfertilised ones. It was thus clear that the small respiration of ertilised eggs must proceed along tracks alternative to the Warburg-Keilin tem, and this has been confirmed by all subsequent work.* Attention must e be drawn to the parallel cases of the orthopteran embryo in diapause 578), the ventral ectoderm of the amphibian gastrula (p. 198) and the yeasts igh and low fermentation rates (p. 496). Whatever the damping mechanism efore fertilisation, it seems to break down in ageing unfertilised eggs, where found that the respiration rises (see p. 572), and in these cases the sensitivity O and KCN rises at the same time. Runnström regarded the cytochrome lase as unsaturated with its substrate in unfertilised eggs — this concept will quite integrate with our present knowledge. More probably the substrates, ydrogenases and cytochrome are held apart.

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* KCN of course inhibits cell-division. Brinley[1] has made the interesting claim that a con tration of KCN which will retard cell-division if the eggs are placed in it will not do so if micro-injected into them.

n the presence of dimethyl-*p*-phenylene-diamine, however, the respiration of unfertilised and fertilised eggs was greatly raised (by many hundreds per), and now the inhibition by KCN and CO was of the same order. This was t we should expect, since the Warburg-Keilin system would, after the addition his substance, be working at full pressure. Runnström[10] drew the right conion that there could be, at fertilisation, no change in the concentration or ity of the enzymes of the Warburg-Keilin system, but rather a change in r accessibility.

the presence of methylene blue, an acceleration of respiratory rate was rved[10] on both unfertilised and fertilised eggs, but much greater in the case he former than in that of the latter. Hence in the former the Warburg-in system, which is side-tracked by reversible dyes, is much more of a ting factor than it is in the latter. The anaerobic reduction of methylene was as active before fertilisation as afterwards, showing that no change in dehydrogenases occurs at fertilisation.†

unnström[7] also studied the effects of abnormal osmotic pressures. Here the lts are hard to interpret. Hypotonic media, for example, depress the respira-of fertilised eggs, but accelerate that of unfertilised ones.

e study of dimethyl-*p*-phenylene-diamine was continued by Runnström[14] rström[1]. At $3 \times 10^{-3}\,M$ the respiration of unfertilised sea-urchin eggs reaches vel of fertilised ones, but they do not become activated. At $2 \times 10^{-2}\,M$ the is maximal on both. Hydroquinone can act in the same way as dimethyl-nylene-diamine. The Swedish authors were of the opinion that both these ances could be reduced by the cells as well as oxidised, so that they would

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hould be stated, however, that Lindahl[9] believes he has shown that the respiration which ds in the presence of CO and KCN is no part of normal respiration. He thinks that the of the normal respiration is abolished and that a new quota appears, the same as that which other circumstances we call the acceleratory effect of CO and KCN. This interpretation is yet generally accepted.

this is contested by Ballentine[1], who believes that he has shown, with the ferricyanide d, a very large change in dehydrogenase activity on fertilisation; about equivalent, indeed, total rise in oxidations. A large increase in the reducing power of the eggs at fertilisation agree with the observation of Dulzetto ten years ago that fertilised eggs give a much stronger tive test for reduced glutathione than unfertilised ones. Ballentine[3] also states that the rogenase activity is associated with granules as well as with cytoplasm as may be seen by ng centrifugally fragmented eggs.

function as a side-track for the dehydrogenases (see diagram), but it is not pr
able that this view is correct.

Already in 1929 Barron[1], following the pioneer experiments of Harrop
Barron with mammalian red blood cells, had shown that the respiration
starfish and sea-urchin eggs was greatly increased by methylene blue, and t
this extra respiration was insensitive to KCN. This was confirmed on unfertili
Nereis eggs, fertilised *Arbacia* eggs, and unfertilised *Asterias* eggs by Barron
Hamburger using toluylene blue. Then Creach found that methylene blue wo
also accelerate the respiration of adult echinoderm tissues, and Friedheim m
the interesting discovery that hallachrome (see p. 406) and echinochrome,
pigment of echinoderm eggs themselves (obtained crystalline by Ball; E_h d
by Cannan), would increase the respiration of echinoderm eggs some sevent
times.* Barron & Hoffman established that the catalytic power of a dye depe
on its position on the E_h scale; those with low potentials (very difficult to oxid
or those with high ones (very difficult to reduce) will not act as respirat
catalysts.† In the proper range comes pyocyanin, the pigment of *B. pyocya*
(Friedheim & Michaelis; Michaelis, Hill & Schubert), and this, tried by Ru
ström[14] on *Arbacia* eggs, behaved in every way similarly to methylene blue.
was expected the increase in the unfertilised eggs was greater than in the fertili
ones (210 against 96 %), and the dye can also catalyse the respiration of cytoly
egg-breis (see p. 493). The further interesting fact was revealed that althou
the respiration of fertilised eggs can be restored to normal or even above it
pyocyanin after inhibition by KCN, the block on segmentation and developm
is not removed thereby. This was confirmed by Korr.‡ It may therefore
accepted, and it is quite significant because, as we shall see when the respirat
of grasshopper embryos is discussed (p. 578), the progress of active developm
is associated with ferrous respiration. It almost looks, therefore, as if non-ferr
respiration cannot be geared to morphogenesis.

In the work just mentioned Runnström[14] found an acceleratory effect of K
on the respiration of the unfertilised egg. The study of this phenomenon and
acceleratory influence of CO was taken up by Örström[2], who was able to m
a model of the state of affairs in the egg by using yeast. The auto-respiration
yeast and its respiration in the presence of formate was, he found, accelerated
CO, but its respiration in the presence of all other substrates was inhibited.
suggested elsewhere (p. 496), this action is probably the result of removal
inhibitory heavy metals by KCN or CO. The fact established by Lindal
namely that the acceleratory effect is photosensitive, like the well-kno
inhibitory effect, may not be in contradiction with this view. Lindahl also fir
by a study of the respiratory quotient, that the acceleration is not due to oxidat
of the CO itself.

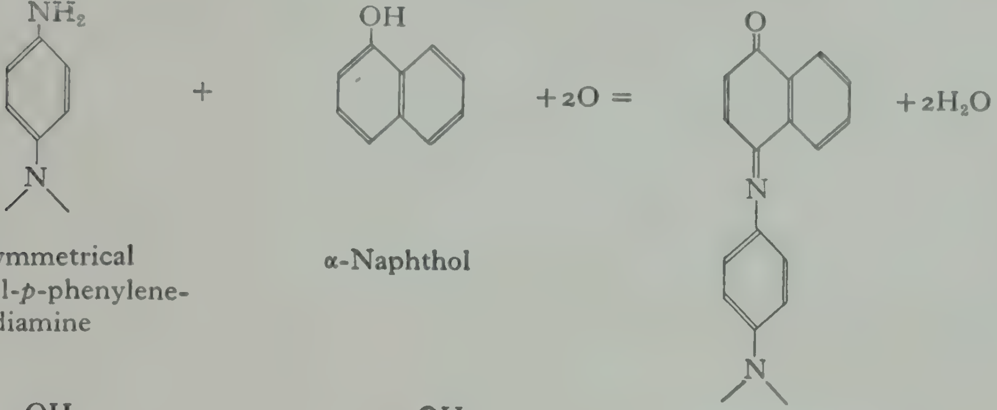
---

* This action of echinochrome is contested by Tyler[8].

† In the light of this (confirmed by Ellis using KCN simultaneously) it is hard to know wh
make of the stimulus to respiration and lactic acid production in echinoderm eggs given by
such as janus green, which should not accelerate respiration, in van Herk's experiments.

‡ Though M. M. Brooks and Ellis have mentioned experiments to the contrary.

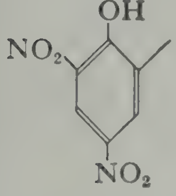
Stimulation of respiration by pyocyanin was combined with temperature terations by Korr[1] in a study of the temperature coefficients of "ferrous" and non-ferrous" respiration. He first confirmed the finding of Rubenstein & erard[2] that the temperature coefficients of pre- and post-fertilisation respiration ffer, so that the rise on fertilisation would cease to exist at high temperatures.



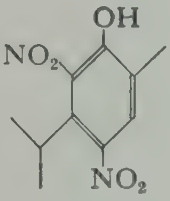
Unsymmetrical dimethyl-*p*-phenylene-diamine

α-Naphthol

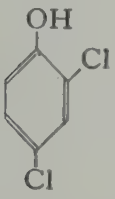
Indophenol blue



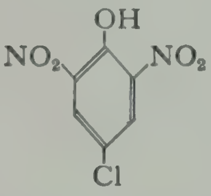
4 : 6-Dinitro-*o*-cresol



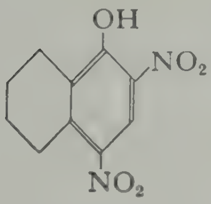
4 : 6-Dinitro-carvacrol



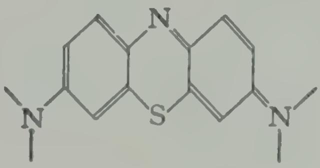
2 : 4-Dichlorophenol



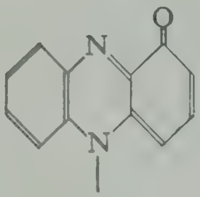
2 : 6-Dinitro-4-chlorophenol



2 : 4-Dinitro-*ar*-tetrahydro-α-naphthol



Methylene blue



Pyocyanin

e then showed that after fertilisation the temperature coefficients of normal spiration and of respiration reduced to low levels by KCN and restored to the ɔrmal level or above by pyocyanin (i.e. a respiration of non-ferrous type), were ɩe same. But in the unfertilised egg the temperature coefficients are a function the pyocyanin concentration, i.e. of the respiration rate. All this strengthens rther the conclusion that in the unfertilised egg the mechanisms for the transfer of activated hydrogen from the substrates to the Warburg-Keilin system are t limiting factors. It should be noted, however, that the fertilised egg in which the Warburg-Keilin system has been thrown out of gear is not identical with the unfertilised egg, even though their respiratory rates may be raised to the same level by pyocyanin. Korr[3] considered that the damping mechanism in the unfertilised egg is best pictured as the masking of cytochrome in some inactive form. But it is doubtful, as a result of work now to be discussed, whether the cytochrome of the unfertilised egg can really be regarded as inactive.* In any case, it is clear that the change at fertilisation will probably not be explicable without recourse to colloidal and protein factors.

The action of the nitrophenols on cell-metabolism and cell-division in echinoderm eggs has been the subject of exhaustive investigations (Clowes & Krahl[3]; Krahl & Clowes[1,2]). Clowes and his collaborators discovered the remarkable fact that at the concentration of dinitro-*o*-cresol which produces the maximum stimulatory effect on the respiration of the fertilised *Arbacia* egg (8×10^{-6} *M*), cell-division is completely inhibited. At lower concentrations the respiration-stimulating effect is of course less marked, and the block to cell-division less complete. At higher concentrations the respiration-stimulating effect also falls off, but the block to cell-division continues unchanged. Nevertheless, this block is completely reversible at all concentrations except the highest, unlike that produced with maximally respiration-stimulating amounts of the reversible oxidation-reduction dyes. KCN, which reverses the stimulation of respiration, acts additively on the cell-division block (Clowes & Krahl[3]). These effects were shown to be obtainable with a wid variety of compounds of nitrophenol type. Fig. 308 shows data for 4 : 6-dinitr carvacrol; at the concentration which increases the respiration of the eggs nearl fourfold, the cell-division frequency has fallen to nearly zero. With rise temperature, the respiration-stimulating effect is decreased but the cell-divisio

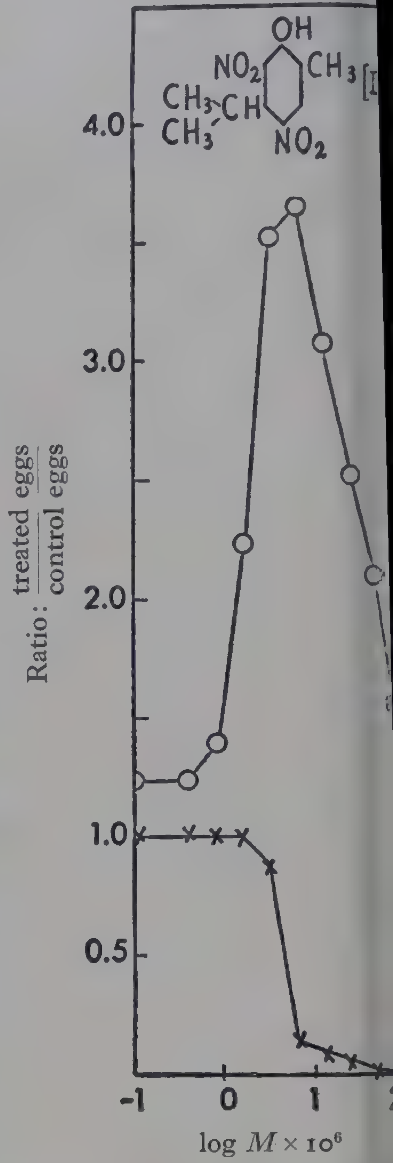


Fig. 308. Stimulation of oxyge consumption and block to cel division of fertilised *Arbac* eggs by various concentratior of 4 : 6-dinitro-carvacrol.

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* Moreover, Korr[4] later revised this opinion, coming to the view that the limiting factor in t unfertilised egg is the provision of the necessary substrates for the dehydrogenases. These them selves, however, increase in activity on fertilisation (Ballentine[1]). One of the puzzling features the situation is that those who examine the echinoderm egg directly for cytochrome (Krah Keltch & Clowes[2]; Ball & Meyerhof) find only the minutest traces there. There is plenty cytochrome oxidase, however (Krahl, Keltch, Neubeck & Clowes), and flavin-adenine dinucle tide is present (Krahl, Keltch & Clowes[3]).

ɔck is unimpaired. The nitrophenols are active at much lower concentrations ˌan the reversible dyes (about 10^{-6} instead of 10^{-3} M). In their presence e respiration of the unfertilised egg can never be brought to equal that of the ·tilised egg, as is the case with methylene blue and dimethyl-*p*-phenylene- ımine. But in another paper Clowes & Krahl[1] found that the effect of the ːrophenols is relatively much greater on the unfertilised than on the fertilised gs.

Krahl & Clowes[3] extended the series to dihalo- and trihalophenols, such as 4-dichlorophenol or compounds such as 2 : 6-dinitro-4-chlorophenol, with e same results. Monohalophenols do not show the effect while the *m*- and mononitrophenols do; but symmetrical trihalophenols do while symmetrical nitrophenols do not. Hydroxybenzoic acids and hydroxybenzaldehydes are ıctive, as also phenols with one aldehydic group (Keltch, Clowes & Krahl); appears that an active compound must have one phenolic hydroxy group and e or more nitro or halogen group. The activity may be much increased by the ːertion of an additional ring, as in the compound 2 : 4-dinitro-*ar*-tetrahydro- naphthol, which exerts its maximum activity at concentrations far below $^{-6}$ M.

A good deal of argument has centred round the mechanism of action of these mpounds. While it is not disputed that the ultimate effect is on the oxidation d reduction of cytochrome, experiments with different CO$_2$ tensions led to the ːw that respiration-stimulation is favoured by a high concentration of the ions within the cell, whereas the cell-division block is favoured by a high ncentration of the undissociated form (Krahl, Keltch & Clowes[1]; Krahl, Clowes Taylor). The effect of *p*H has proved difficult to interpret (Krahl & Clowes[4]; owes, Keltch & Krahl; Tyler & Horowitz; Hutchens, Krahl & Clowes), d it has not been possible to dissociate very clearly the two effects of the ːrophenols. Whatever the interpretation may turn out to be, these effects are clear-cut and well marked that they must be of importance for the future derstanding of the integration of respiration and morphogenesis.

ˈo the above, it may be added that reagents poisoning copper catalyses (e.g. assium dithio-oxalate, diphenylthiocarbazone, or isonitro-acetophenone) give inhibition of cell-division (Clowes & Krahl[4]). The relations between effect on ɔiration and effect on cell-division may be summarised as follows:

	Effect on respiration at complete cleavage block Normal ≡ 100 %
Low oxygen tension (see p. 564)	35
CO	35
KCN	35
Phenyl-urethane (see p. 517)	100
γ-(2-methyl-piperidyl)-propyl-benzoate	100
Halo- and nitro-phenols	350

l the extra respiration caused by the substituted phenols is sensitive to cyanide d CO (Krahl & Clowes[5]). Though raising the overall oxygen-consumption, ·y must inhibit some intermediate step essential for cleavage. Carcinogenic lrocarbons, tried as their water-soluble choleic acid derivatives (Keltch, Krahl ˈlowes), gave very small stimulating effects on cell-division in *Arbacia* eggs.

Among the most important of the other inhibitors of metabolism which ha been tried on echinoderm eggs are fluoride and iodoacetate (cf. p. 492). A 0·02 solution of the latter did not inhibit fertilisation in *Urechis* (Tyler & Schult and Ellis found a surprising resistance to the action of both in the fertilised eg of *Urechis* and *Strongylocentrotus*, as tested on the rate of cell-division. In 0·01 iodoacetate there was no slowing of the division rate. By direct estimations the glutathione content of the developing eggs, Ellis was able to prove that t iodoacetate penetrated and combined with the sulphydryl groups. At the sar time there seems no reason why development should not proceed at the exper of some substrates other than those involved in glycolysis. Runnström[17], usi 0·03 *M* iodoacetate on *Arbacia* eggs, found no inhibition of fertilisation, althou there was a 55 % inhibition of respiration and only the earlier segmentatio could be passed through. Pyruvate reversed the respiratory inhibition, but cou not restore normal morphogenesis.

From the work of Simon[2] it appears that radium emanation has little action the respiration of the egg, judging from experiments on the piddock clar *Barnea candida*. But L. Chesley, using X-radiation on *Arbacia* and *Chaetopteru* obtained interesting results showing that developmental anomalies appear doses quite insufficient to affect the respiration either normal or intensified b methylene-blue catalysis.

One of the most obscure phenomena about the early development of th echinoderm egg is the acid production which occurs shortly after fertilisatio At Runnström's suggestion, Ashbel[1] discovered in 1929 a considerable positi pressure produced in manometers by eggs after fertilisation under anaerob conditions. We know now that this takes place also under aerobic conditio (Borei[1]; Laser & Rothschild) and the fixed acid produced amounts to 8 mili equivalents per c.c. eggs. Unfortunately success has not attended efforts ascertain what this fixed acid is. The acid production of eggs cytolysed b hypertonic or hypotonic sea-water was found by Runnström[12] to be increase by hexosemonophosphate, suggesting glycolysis, but it was not inhibited b iodoacetate, and when cytolysis was produced by freezing and thawing, hexos monophosphate had no effect (Runnström[16]). With saponin cytolysis, phloridzi inhibited the acid production (Rothschild). The position thus needs furth elucidation.

Before fertilisation, as we have seen, the respiratory rate of the sea-urchin eg is low. On ageing (i.e. if no fertilisation takes place) the respiratory rate gradual rises, and the check to ferrous respiration must slowly be released, as sensitivi to KCN and CO also begins. Ageing is known to be accompanied by permeabili changes (Goldforb). The actual length of the fertilisable period is variab according to the species, but always shorter than the time elapsing befo the capture of external nourishment by the embryo if fertilisation had occurre The aged egg dies, therefore, in possession of plenty of raw materials capab of supporting life (cf. pp. 258, 511). According to the older Lillie-Ju “fertilisin” hypothesis (F. R. Lillie[5]; E. E. Just[2]), fertilisability ceased becaus the substance which attracts spermatozoa (“fertilisin”) ceases after a time

fuse out from the eggs. But this does not explain why the eggs should die.
yroxin was found by Carter to extend considerably the fertilisable life
echinoderm eggs; he believed that it was related chemically to "fertilisin"
e p. 214). The fertilisable life is also known to be extended by anaerobiosis
KCN. To these factors Whitaker[13] has now added 1 % ethyl alcohol, which
ends the fertilisable life of *Urechis* eggs 300 %, while 1 % glucose extends it
n longer. It seems likely that these substances act nutritionally, for it may
ll be that the yolk reserves are not accessible to the egg until after the colloidal
anges occurring on fertilisation. Tyler, Ricci & Horowitz have shown that
ne of the rise in respiration can be accounted for by bacterial growth. Sterile
nditions prolong the fertilisable life of eggs enormously, but the alcohol at
hitaker's concentration does not act by virtue of its bactericidal properties.
ler & Dessel say that acid pH prolongs the life of gephyrean eggs. Here again,
en, is a subject which needs much further elucidation.

An important advance was that made by Shapiro[4] in his studies on the respiratory
es of fragments of sea-urchin eggs (*Arbacia*) obtained by centrifugation
erogons; see Fig. 211). By strong centrifugal force the eggs were broken into two
ves, the upper (light) one white, containing the nucleus, hyaline cytoplasm and
-cap; the lower (heavy) one red because of the echinochrome granules, and
ntaining most of the yolk. The light half is a little larger than the heavy half
l. 116,750 cu.μ against 84,700 cu.μ). Manometric measurements of re-
ratory rate then gave the following figures:

	μl. O_2/10 c.mm. cells/hr.	
	Unfertilised	Fertilised
Light halves	1·23	3·49
Heavy halves	2·81	2·66
Whole eggs	1·49	3·85

us the unfertilised light half, containing nucleus but no pigment, respired at
roximately the same rate as the whole egg, while the unfertilised heavy half,
hout nucleus but containing yolk and echinochrome, respired 88 % in excess
. The combined activity of both halves, allowing for volumes, was thus 29 %
ater than that of the intact egg. On fertilisation, only the light protoplasmic
es showed the well-known increase, and the combined activity of both halves
r fertilisation was thus 17 % less than that of the intact egg.

hese experiments are suggestive in that they associate the "damping"
chanism already described with the protoplasmic part of the egg. The heavy
ves produced by centrifuging seem to have escaped from its influence.
mination of the action of inhibitors and stimulators of metabolism on the
ves will be awaited with much interest. In the meantime Navez & Harvey
e given a short report on the indophenol oxidase in fragmented eggs, from
ich it seems that there is twice as much of this fundamental enzyme in the
vy halves as in the light ones, as detected by the histochemical method.
her workers, however, find that the increase of respiration due to para-
nylenediamine is more marked in the light than in the heavy halves, (Boell,
ambers, Glancy & Stern).

We come now to a remarkable series of papers by Tyler on the energetics differentiation, which show a consciousness of the most essential probler namely the integration of metabolism with morphogenesis. In the first of the Tyler[2] compared the oxygen consumption of "half" and whole embryos of t sea-urchin, basing his argument on the view that when two blastomeres a separated and two identical but smaller embryos develop from an egg whi would otherwise have produced only one, there is twice as much morphogenet work going on for the same amount of energy sources (raw material) as normall Using *Echinus microtuberculatus*, measurements of respiratory rate (per mgr nitrogen) showed that it was identical in cultures of normal embryos and dwa ones. But as has long been known (Driesch[2] for the sea-urchin; Spemann Falkenberg for the newt; Morgan[6] for *Amphioxus*; Tyler[1] for *Chaetopterus*), dwa embryos develop with a considerable delay. Though it has been classical to pu this down to the exigencies of "regulation", Tyler suggested that it was real due to the necessity for the performance of twice the usual amount of morphogenetic work. In his own experiments the delay was of the order of 38 %. order to reach a given stage of development, therefore, for example the end gastrulation, the dwarf embryos actually use considerably more oxygen than th normal ones, i.e. two dwarf embryos require more than one normal one althoug the amount of living matter is the same.

Unfortunately these interesting experiments teach us relatively little about th metabolic cost of morphogenetic work, for the variables of maintenance an growth are not really ruled out.* In order to keep itself going as a physica system quite without any sort of differentiation, a cell has to expend energy, an since the time taken to reach the given morphological stage was longer, mor maintenance must have been required.† And we do not know the indispensabl datum, namely, what the respiratory rate would have been during the extra tim required to reach the same stage, if the maintenance quota had been subtracte from it. There is also the factor of growth. This is not eliminated in experimen with echinoderm eggs, since no egg is absolutely "alecithic", and as the embry develops, the yolk, however modest and transparent, will be converted int respiring protoplasm. Hence the rise in respiratory rate during echinoder development *CE* (Fig. 111).

Nevertheless, Tyler's interpretation of regulation is acceptable enough. In later paper[4] he showed that giant embryos obtained by fusion of normal egg

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* Moreover, the energy needed for the formation of new surfaces is usually grossly overrate Borsook has calculated, using the equation

$$T\left(\frac{\partial Y}{\partial T}\right)_{\sigma} = Y-\left(\frac{\partial H}{\partial \sigma}\right)_{T},$$

where T, Y and σ refer to absolute temperature, surface tension and surface area, that to mak 10^6 sq. cm. of surface needs only 3 gm. cals. even if Y is taken as high as for an air-water interfac i.e. 72 dynes/sq. cm., though for living cells it is more like 2 dynes/sq. cm. If the volume of th chick embryo is taken as 50 c.c. and all its cells assumed to be $\frac{1}{10}$th the volume of erythrocyte then even if it were a solid cell-mass, the total surface would only be $2{\cdot}4 \times 10^5$ sq. cm. Yet th total energy turnover of the chick embryo during its development is some 23,000 gm. cals.

† This criticism does not seem to me to be met even in Tyler's recent, otherwise very instructiv monograph[7].

f. p. 103) develop even faster than normal-sized ones. This had previously been served by Spemann & Wessel-Bautzmann on the newt.

In a third paper[4], Tyler studied the effect of temperature on cleavage rate and e rate of gastrulation and pluteus formation, in an attempt to dissociate cleavage id organogenesis, on the echinoderms *Dendraster*, *Lytechnis* and *Strongylo-* *ntrotus*, the ascidian *Ciona*, and the gephyrean worm *Urechis*. It proved npossible, however, to do so. With the same species an attempt to dissociate ygen consumption and developmental rate (Tyler[5]) also failed, but the results e a striking confirmation and extension of the earlier ones of Ephrussi[4], namely at the total oxygen consumption of the eggs of invertebrates throughout their velopment is independent of the temperature. If membrane elevation is evented in sea-urchin eggs, gastrulae are formed the walls of which have nearly ice the normal thickness, and there is some delay at gastrula and prism stages. evertheless, the rate of respiration was found to be exactly the same (Tyler[6]). ut the same arguments apply in this case as in that first discussed above.

As additional proof of the linkage (obscure though its mechanism may be) tween respiration and development, Tyler & Horowitz[2] compared the respira- on of *Urechis* eggs normally fertilised with others parthenogenetically stimulated such a way as either to produce cleavages or not. In all cases the respiratory te rose with time, but more after fertilisation than after parthenogenesis, and ore during parthenogenetic cleavage than non-cleavage. Urethane reduced the te of the fertilised eggs to a rate more approaching the others.

The last question which arises is whether it is possible to distinguish any cillations of oxygen consumption during the phases of cell-division itself. It s been usual to deny this (*CE*, p. 641), and scepticism on technical grounds is be recommended, but Runnström[13] has brought forward some experiments nich seem to show a periodic variation during the first three cleavages, and Reiss supports this by periodic kicks on the E_h readings of a platinum electrode an egg suspension. Moreover, Trurnit, using delicate thermocouples adjacent echinoderm, and actually *inside*, amphibian, blastomeres, claims to have found eatable peaks of heat-production, the rise beginning when nuclear cleavage gins, and attaining its maximum just before the moment of cell cleavage.

The respiratory quotient of *Urechis* eggs during development has been asured by Horowitz[3]. It falls rapidly from unity at 2 hr. after fertilisation to at 20 hr. This agrees with the succession of energy-sources seen in so many ner organisms, but is remarkable in that the fall in R.Q. begins directly from tilisation. It could not be arrested by adding glucose to the medium. This may the place to mention a curious respiratory pigment found in the eggs of the ne gephyrean worm by Horowitz[2]. "Urechrome" changes from pink to yellow versibly on oxygenation, and its rH was measured. Since it occurs naturally in th forms its physiological function is particularly obscure (cf. the pigment entioned on p. 210).

To complete this section we must glance at work done on some non-marine rms. Baldwin[3] has given us a careful study of the respiratory quotient during development of a pulmonate gastropod, *Limnaea stagnalis*. The measurements were made, after shelling out the eggs from their capsules, by the Dickens-Šin annular cup manometric method. The R.Q. continued at approximately un throughout development, but since a synthesis of fat takes place in these for (as was first shown in the remarkable pioneer experiments of F. G. Burdach 1853 and confirmed by Baldwin), the catabolic utilisation of protein and which probably goes on, at any rate towards the end of development, was mask The quotient is nevertheless of interest in view of the large stores of pol saccharides in molluscan eggs (see p. 29).

Another piece of work on molluscan eggs is that of Leiner[4], who found a ve marked decrease in carbonic anhydrase activity during the development *Aplysia*, although the respiratory rate was constantly rising. One may have the suspicion that this is connected with the formation of the alkali reserve, referred to below (p. 585).

Additions to our knowledge of the metabolism of cestode eggs have been made by Friedheim & Baer. The metabolism of the adults, as is well known, is very peculiar, volatile fatty acids being given off as end-products (cf. the recent reviews of Krüger[3]). Friedheim & Baer studied the cestode *Diphyllobothrium latum*, which lives in the intestines of man, the dog, cat and fox. The eggs (see p. 30) develop free in water, and the first ciliated larva is transformed into a second larval form in the gut of the fresh-water crayfish. On passing along the food chain into fishes such as the pike, the worm lies inert in the muscles as a "plerocercoid", and at last is eaten by mammals. Investigation show that the eggs are obligate aerobes though the larvae and adults are facultative anaerobic. The following data are therefore of interest:

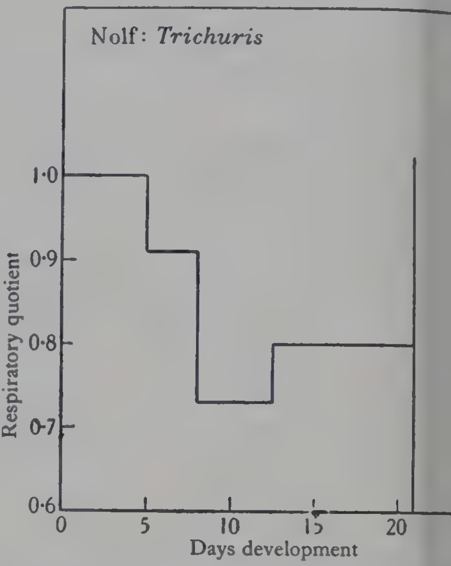

Fig. 309. Respiratory quotient duri the development of the nemato embryo (*Trichuris trichiura*).

	Oxygen uptake	Q_{O_2}	$Q_L^{O_2}$	Q_L^{N}
Eggs (dry weight not measured)	23 c.mm./hr.	—	None	No
Plerocercoid	—	− 0·55	+0·28	+1·
Adult	—	− 2·36	+1·75	+5·
Adult + glucose	—	−15·01	—	—

The adult data agree with other observations on the tapeworm *Moniezia expan* by Alt & Tischer. The most peculiar characteristic of the metabolism of the egg however, was that it was completely inhibited by KCN but not at all by CO a unique case. That of the adults was inhibited 60 % by KCN but not by C No explanation has as yet been offered for this, but the phenomenon is probab general to cestodes, as the pike's tapeworm *Triaenophorus lucii* also shows it.

A nematode, *Trichuris trichiura*, has afforded data on the R.Q. througho development in the work of Nolf. As Fig. 309 shows, it declines steadi This is the first instance of several (p. 603) in which there is apparently utilisation of carbohydrate preceding the utilisation of protein and fat.

)n the eggs of the nematode *Ascaris megalocephala* (see p. 139) Huff & Boell :overed the remarkable fact that disorganisation of the cell by ultra-centrifug- causes an inhibition of the ferrous respiration while leaving the non-ferrous piration unchanged. This is shown from the following figures:

	c.mm. O_2/hr./10^6 eggs	
	Alone	+KCN $10^{-3}M$
Control eggs	−102·3	−11·3
Centrifuged eggs	−22·3	−12·8

vill be seen that this is very significant in view of what has already been said ut the association of non-ferrous respiration with the low metabolism of the ertilised echinoderm egg, and what will later be adduced about its association h the low metabolism of orthopteran diapause. The idea is beginning to stallise that ferrous, cyanide-sensitive, respiration is more connected with mal colloidal conditions and more closely linked with morphogenesis than ı-ferrous respiration. The latter may support life, however, in "damped" iods or regions. All the eggs of Huff & Boell's experiments subsequently lerwent normal cleavage.

n stages of the life cycle later than the embryo, the metabolic rate of inverte- tes, though poikilothermic animals, falls (see *CE*, p. 746, and new confirmation Davis & Slater on the earthworm, Chapheau on the oyster's hepatopancreas, Terao on daphnids). The subject will be alluded to again later (p. 600).

·32. Respiration of the eggs of Arthropods

)n the embryos of crustacea there is only one piece of work to be recorded, which measurements were made of the respiratory quotient during the elopment of the shore-crab *Carcinus* *ıas* (Needham[11]). In the cleavage states approximately unity, but by the time yolk has diminished to four-fifths the ıeter of the egg, it has fallen to 0·72, which there is a slow rise to about 0·8 Fig. 310). Clearly over the greater part ıe developmental period there is a com- ion of protein and fat, with a predominant oustion of carbohydrate in the earliest :s. This is the second case of the succes- of energy sources, carbohydrate-protein- which we have noticed; another will tly be adduced. Incidental to this work the interesting finding that normal respiration and normal metabolism (so s the R.Q. shows it) would go on in the embryos of this crab at temperatures e the thermal death-point of the adult.

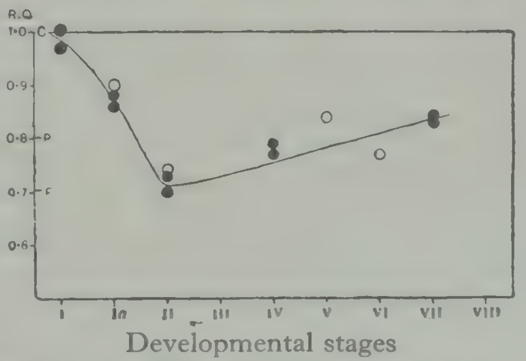

Fig. 310. Respiratory quotient during the development of the shore-crab embryo (*Carcinus moenas*). ● at 37 ; ○ at 15 . *C*, *P* and *F*, the theoretical quotients for normal combustion of carbohydrate, protein and fat.

c come now to the main subject of this section, the advances which have been made in the study of respiration and metabolism in insect embryos. owe in particular to the work of J. H. Bodine and his collaborators in I one of the most comprehensive and elaborate accounts of the embryonic m bolism of an important group of animals existing in the literature. What foll applies mainly to the grasshopper *Melanoplus differentialis*, but many ot species of orthoptera were studied by this school, and the same description, w minor modifications, holds good for all of them. The peculiarity which affor the means for an unusual insight into embryonic metabolism was the "diapaus

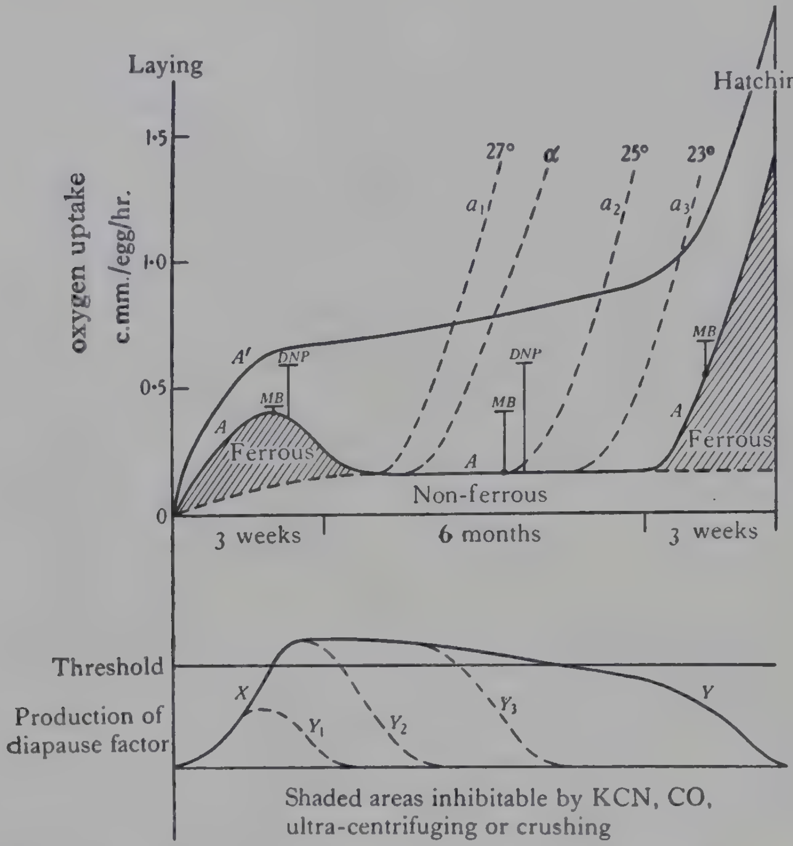

Fig. 311. Diagram illustrating the mechanisms of respiration and diapause in the grasshop (orthopteran) embryo (see text). *MB*, Methylene blue; *DNP*, dinitrophenol.

After 3 weeks' development the egg of the grasshopper enters a dormant sta which may last for many months, and at the conclusion of this diapause, devel ment proceeds as before, culminating in hatching after a second 3-week peri It is known that during diapause there is a complete cessation of mitosis, grow yolk utilisation, and movement (Slifer[2]).

Bodine[3] and Burkholder first studied the respiration of the eggs during th periods. As shown diagrammatically in Fig. 311, the respiratory rate first ri to a maximum (curve *A*), then falls to a low and constant level during diapau finally rising again very rapidly during the last three weeks. Cumulative respiration would follow a line such as *A′*. Now it had long been known t diapause could be artificially broken at any time by exposing the eggs to temperature about freezing-point. On returning to room temperature they wou then set about the concluding phases of their development and hatch in th eks (as represented by curve α in the diagram). On the other hand, if the eggs re maintained at relatively high temperatures directly from the time of laying, n also they would complete their development in a shorter time (according to rves a_1, a_2, a_3); the duration of diapause, but not its existence, being dependent on external temperature.* Bodine[5] tried to explain these facts by the assump- n of a substance which inhibits development on attaining a certain threshold rve X in Fig. 311). The concentration of this substance at any given time uld be the resultant of processes of formation and destruction. Exposure to 0° m the time of laying prevents diapause altogether. We may say, therefore, t the destructive process has been differentially favoured at the expense of formative process, and so no threshold concentration ever arises (curve Y_1 the diagram). On the other hand, exposure to 0° at a later date, after pause has set in (i.e. the threshold concentration has been reached) will break diapause; here again the destructive process must be less susceptible to cold ve a lesser temperature coefficient) than the formative one and consequently concentration of the substance will fall below the threshold (curves Y_2 and Y_3 the diagram) and development will proceed as soon as room temperature is ained. Such an explanation follows the general lines of Goldschmidt's thought e p. 400). It would be satisfying if some definite evidence could be found, e.g. control of diapause by injection of extracts of other embryos.†

It should be emphasised at this point that there are no morphological differences ween embryos in late pre-diapause, diapause, and early post-diapause; they only be distinguished by their rate of oxygen-consumption.‡ At all stages oxygen consumption can be reversibly depressed by exposure of the embryo hypertonic solutions (Bodine[4,5]; Thompson & Bodine[5]; Bodine & Thompson; hlipper); on return to isosmotic conditions, the respiration returns to a level or high depending on the original stage of the embryo. Diapause block can erefore not be affected by this means.§

discovery of real importance was made when Bodine[7] and Bodine & Boell[1,2] wed that the respiration of the grasshopper egg was sensitive to KCN and CO when in the actively developing stages, and furthermore that the fraction of iration insensitive to KCN and CO in these stages was of approximately the e order as the whole of the respiration during diapause. These facts are shown he hatched areas on Fig. 311. Bodine & Boell[2] drew attention to the parallel veen this case and that of the unfertilised and fertilised echinoderm egg (see p. 199). There is thus a non-ferrous respiration (see diagram on p. 578) running

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his is the distinction between diapause and hibernation. Hibernation is caused by low perature; it is not inherent, as shown by Bodine[1] on the nymphs of another grasshopper, *rtophaga viridifasciata*. True diapause exists, between laying and the initiation of development, nany other invertebrate groups, such as daphnids (where distinct races exist, one showing it another not; T. R. Wood) and brine-shrimps.

arlier theories postulating intoxication by metabolic waste-products may be disregarded.

.g. the following figures taken from the later work of Bodine & Boell[9]; for an embryo weighing 7 mgm. Q_{O_2} is in pre-diapause −1·52, in diapause −0·59, and in post-diapause −1·47.

ne differences between the temperature coefficients of diapause and developing embryo iration (Goodrich & Bodine) are not very marked.

throughout development in the orthoptera, and in addition a respiration ferrous type exclusively linked with active morphogenesis. Bodine & Boe also found that during diapause there is a certain stimulation of respirati by CO, analogous to the Örström effect on the unfertilised echinoderm e (p. 496). Inhibitions by KCN cause delays in hatching time (Robbie, Boell Bodine).*

From inhibitors they turned to stimulators with equally satisfactory resul Before dealing with these, however, it will be convenient to refer to Boell's stu of the normal R.Q. during the development of the grasshopper. This is shown Fig. 312. Beginning at just under unity, it falls to a minimum at 0·6 during t pre-diapause period but is stabilised at 0·7 before diapause has started. This val is maintained, apart from slight oscillations at the beginning of the post-diapau period, till hatching. Thus we have a third case suggesting the carbohydrat protein-fat succession of energy sources. Some 75 % of the total oxygen co sumed corresponds with the loss of fat actually found by Slifer[1,4].

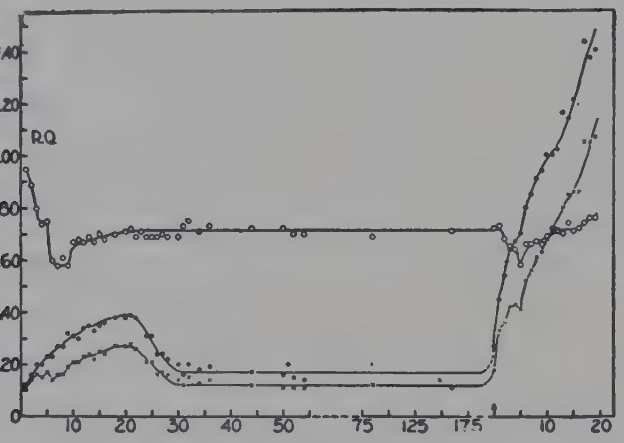

Fig. 312. Respiratory quotient during the development of the grasshopper embryo (*Melanop differentialis*). • O_2 uptake (c.mm./egg/hr.); × CO_2 production (c.mm./egg./hr.); o R.Q. T arrow indicates the end of diapause.

The action of methylene blue (Bodine & Boell[8,10]) differs according to the sta at which it is applied. During the actively developing pre-diapause the stimulati of respiration is 2 %, during diapause it is 101 %, and during post-diapause is 24 %. These results, indicated in Fig. 311, show, as would be expected, that it possible to side-track the Warburg-Keilin system far more effectively when it out of action during diapause than at other times. It should also be noticed th just as in other cases (pp. 199, 568) the final level is much the same since t stimulating action is much greater on the phase originally damped.† All the effects hold good for the isolated embryos as well as the intact eggs, general speaking (Bodine & Boell[9]). Bodine & Boell[11] also studied the effects of 2 : dinitrophenol and 3 : 5-dinitro-*o*-cresol. Here again the stimulation was one 275 % on diapause embryos and of 150 % on developing embryos (see Fig. 31

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* Purely morphological effects of KCN on beetle embryos have been described by Brauer.

† In later papers Bodine & Boell[10] have stated that the respiration stimulated by methylene blue sensitive to CO though not to KCN. This very odd finding requires elucidation (cf. the work Friedheim & Baer above, p. 576).

ging the final levels rather close. As in other cases (p. 571) the excess respira-
was fully sensitive to KCN and CO. This demonstrated that the Warburg-
lin system *can* be made to function during diapause, and suggests that it is
tivated either by colloidal changes or by some inhibition which the nitrophenols
overcome.* Bodine & Boell[11] then noted that the R.Q. of the nitrophenol-
ulated respiration was different from the normal; instead of being 0·7 it was
veen 0·91 and unity. Investigating this phenomenon they were able to prove
since neither iodoacetate nor fluoride would inhibit it, and since considerably
e ammonia was produced by the embryos in presence of nitrophenols than
mally, it was due to the catabolism of protein ending in ammonia. This was
ase parallel, therefore, to the fluoride-inhibited chick embryo observed
Needham[8] (see p. 592). And it pointed to the interesting conclusion that
ough the grasshopper embryo during the latter part of its development is
busting fat, it is nevertheless quite capable of combusting protein if circum-
ces make it necessary that it should do so. In another paper, Bodine &
ll[7] further showed that post-diapause embryos *in vitro* can still utilise added
ose.

nother instructive line of attack employed by Bodine & Boell[5] was ultra-
trifugation. By means of the air ultra-centrifuge the grasshopper egg can be
tified into lipoidal, protoplasmic and proteinaceous layers, and if the rotational
e does not exceed 20,000 g. recovery and development are possible. Now the
ortant fact emerged that although in pre-diapause or post-diapause eggs
es of only 1300 g. will decrease the respiratory rate considerably and forces
0,000 g. will lower it to about 50 % of its normal value (i.e. nearly to the level
he non-ferrous fraction), forces of even 400,000 g. have no effect on the
iration of diapause eggs. The ferrous fraction is therefore sensitive to cell
rganisation while the non-ferrous fraction is not. We have already seen a
ilar case in the experiments of Huff & Boell on the nematode egg (p. 577).
he other hand, X-rays (Boell, Ray & Bodine) did not differentiate between
arious forms of respiration. At the doses used, diapause respiration was
fected, and developing embryo respiration was only decreased in so far as
development was slowed.

mong other valuable contributions on this subject the following may be
d. The yolk (containing as it does many nuclei and some cells) cannot be
looked as a seat of respiration. During pre-diapause and diapause the oxygen
umption of the whole egg is double and sometimes more than double that
e embryo alone (Bodine & Boell[7]); this corresponds to the extra-embryonic
brane respiration of amniotes. Moreover, at the onset of post-diapause, the
ease in oxygen consumption occurs first in the yolk-cells. Tyrosinase, followed
ugh development (Bodine & Boell[4]), rises to a maximum of activity during
-diapause and remains there throughout the two succeeding periods. It is
tly contained in the yolk. Yet the naturally occurring substrate appears only
e post-diapause period (Bodine, Allen & Boell). By centrifugation, an acti-

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the other hand pyocyanin, studied by Carlson & Bodine, gives 100 % stimulation of
ration, whether in diapause or not.

vator, the action of which could be imitated by sodium oleate, is removed fr the enzyme (Bodine & Allen; Ray & Bodine[2]). During pre-diapause there great decrease in the potency of the naturally occurring lipidic protyrosin activator (Bodine, Ray, Allen & Carlson; Bodine & Carlson[1]). Other proper of the enzyme have been studied by Allen & Bodine[1], and Bodine, Carlson & state that in eggs X-rayed so as to destroy the embryo but not the yolk and ser cells, protyrosinase and its activator are formed much the same as norma X-rays cannot substitute for the activator (Ray), but a number of other age can, such as heat (63–77°), detergents, acetone, urea, urethane, and reductior salt concentration by dialysis. Denaturation of the enzyme precursor theref enters the picture (Allen & Bodine[2]).* The precursor has a different ultra-vi spectrophotograph from the enzyme (Bodine & Carlson[2]).

Indophenol oxidase activity was measured by Bodine & Boell[6] by determini the excess respiration of egg brei in the presence of dimethyl-*p*-phenyler diamine. Actually this should measure the activity of the whole Warburg-Ke system (see diagram on p.565). Where, as in egg-brei, the structure of the cells been destroyed, the respiration was insensitive to KCN and CO, a further proof the dependence of the Warburg-Keilin system on integrity of cell structure. Eg brei from diapause eggs, moreover, respires at the same rate as the intact eg Nevertheless, when optimal amounts of this activated substrate were added, the was a considerable extra respiration quite cyanide-sensitive. A view of t activity of the Warburg-Keilin system, obtained in this and other ways (T. Allen), showed that it resembles the tyrosinase curve in rising somewhat to t end of pre-diapause and then continuing unaltered until post-diapause. But post-diapause it rises considerably until hatching. These experiments demo strate that the intrinsic activity of the Warburg-Keilin system is not diminish during diapause, but only in some way inactivated. It is puzzling, however, th no cytochrome *c* is spectroscopically visible in the embryos.

"Peroxidase" (by the guaiacum test) is absent during pre-diapause, gradua increases during diapause and rapidly during post-diapause (Bodine & Boell But the enzyme is always in the embryo and not in the yolk. Glutathione (qua tative tests) is equally present throughout development (Evans[1]). Bodine Wolkin determined the total iron in yolk and embryo; it passes regularly fro the former to the latter, and the respiratory rate cannot be dependent on it, f embryos with the same total iron concentration show entirely different oxyg consumptions in accordance with their developmental stage. The relation betwe oxygen tension and oxygen consumption does not vary with the stage (Bodine the curve is very similar to that already described for the eggs of echinoderm During diapause, respiration and development are less susceptible to ultravio light (Ray & Bodine[1]) or X-rays (Bodine & Evans[2]; Evans[2]) than at other tim

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* One cannot help drawing attention here to the similarity between this case and the liberation the primary inductor in the dorsal lip of the blastopore.

† Rhythmic pulsations of the body-wall, possibly connected with respiration, and later merg in the heart-beat, occur in orthoptera. Extensive studies have been made on their reaction temperature (Thompson[1]), electrolytes (Thompson[2]), CO_2 (J. F. Walker), etc.

3y way of commentary on the above account it may be said that diapause may ur at any stage of the insect's life cycle* and that exposure to low temperatures by no means the only stimulus which can break it. There is a wealth of omological literature (available through the reviews of Uvarov; Roubaud; usin and others) which supports this. For example, diapause may occur in nphal stages, as in the bug *Reduvius* (Readio) and in some grasshoppers, such *Chortophaga viridifasciata* (Bodine[1]), or in wasps such as the mud-dauber, *liphron caementarium* (Bodine & Evans[1]). What has been said about depression espiratory rate applies, *mutatis mutandis*, to these cases. In the work of Heller[3] the hawkmoth, *Deilephila* (p. 476) we have already seen an example of diapause urring during metamorphosis. Diapause can be broken by many agencies, . cold (see above, also Flemion & Hartzell; Burdick; Bodine & Robbie); tion, electrical or mechanical shocks (Roubaud); various chemicals such as ol and carbon tetrachloride (Pepper); and parasitism (Salt; Varley & Butler). at it is genetically conditioned in silkworms we know from the work of Uda and neya; the characters of the embryo as regards diapause are a case of "maternal eritance".

The silkworm, *Bombyx mori*, formerly a classical object of research, has eived less physiological study than the grasshoppers. Curves for respiratory e in diapause (univoltine) and non-diapause (bivoltine) races, similar to those Fig. 311, have, however, been established for the moth *Lymantria monacha* Tuleschkov and the silkworm by Ashbel[2]. Ashbel[7] finds that in this form the duration of the diapause depends on the temperature. Thyroxin, added egg-powders prepared from embryos at different stages, has a much larger ect upon the diapause material than upon that from either pre-diapause or st-diapause (Ashbel[5]). Neither X-rays nor radium emanation affect the length the diapause (Ashbel[6]). Changes in glutathione (Teodoro; Demjanovsky) are yet clear and the extremely deviant R.Q.'s of Ongaro[2] may be disregarded. tal oxygen consumption after various forms of parthenogenesis by strong ds† in silkworms have been measured by J. Fukuda; the differences were not t (cf. the work of Tyler mentioned on p. 575). Wolsky[4] states that this respira- is insensitive to CO. Kozhanchikov[1] reports that the larvae of the pyralid h *Loxostege sticticalis* are especially resistant to anaerobiosis during their ause, and pile up an oxygen debt which is later discharged at the expense it oxidation.

or accounts of specialised mechanisms for respiration in insect eggs the ler must be referred to the entomological literature. We may mention two or e, however. The egg of *Oöencyrtus johnsoni*, an encyrtid parasite on the eggs e cabbage bug *Murgantia histrionica*, has a special "aeroscopic plate" on its

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he insects are not the only group of animals with embryonic diapause. Something remarkably it occurs in mammals. Hamlett[3] writes: "We know of a dozen mammals whose period of ation is regularly from 2 to 6 months longer than actual embryonic development", e.g. certain gers, bears, shrews, etc. and the stonemarten and pinemartin. The diapause occurs at the tocyst stage preceding implantation (see p. 84). We have already seen (p. 78) that the blastocyst e may be unusually prolonged in most mammals if lactation is occurring. Hamlett gives a l discussion of the endocrine mechanism (progesterone) and the biological significance of nmalian "diapause".

irelli[7] gives an account of Tichomirov's strange discovery, which preceded the work of J. Loeb, parthenogenesis in the silkworm may be brought about by treatment with strong acids such lphuric or hydrochloric.

case to allow of direct respiration, avoiding diffusion through the shell of the egg (Maple). Later, the encyrtid larvae become attached to the host's trac system (Thorpe[1]). The larvae of *Cryptochaetum grandicorne*, parasitic in haemocoele of the coccid *Guerinia serratulae*, have remarkable caudal filame to aid in the absorption of oxygen and nutriment from the host (Thorpe[2]).

3·33. Respiration of the eggs of Fishes

Teleostean fishes alone lend themselves to experimental attack on embryo respiration and heat production. From the morphological details already gi (p. 326) it will be remembered that their eggs are highly lecithic and meroblas cleavage being confined to a small germinal area, and the blastoderm t growing round the yolk so as to enclose it in a yolk-sac, while at the same ti the embryonic axis is laid down. Observations have been made mostly on freshwater forms such as *Salmo irideus* and *Perca fluviatilis*, besides the American marine minnow or killifish *Fundulus heteroclitus*.

As would be expected from the growth of the embryo at the expense of inert yolk, the oxygen consumption of the whole egg rises regularly throughout development (Schlenk and S. Smith[1] on *Salmo*; Trifonova[3]* on *Perca*; Amberson & Armstrong and Phillips[1] on *Fundulus*; Bežler on cyprinids). In postnatal life metabolic rate falls, as in all animals (Raffy on eels; Keys[1] and N. A. Wells on the killifish). More interesting is the respiratory quotient.† Here quite independently two pieces of work (Schlenk and Amberson & Armstrong) have given strong indications of an R.Q. of unity at the beginning of development, followed by a value of about 0·7 for the major part of the period, although t development of *Salmo* studied by Schlenk takes (till hatching) a little over 40 da and that of *Fundulus*, studied by Amberson & Armstrong, is complete in 12. T latter set of data is shown in Fig. 313. Teleostean fishes therefore constitu yet another case of an apparent succession of energy sources, carbohydr preceding protein and protein preceding fat.

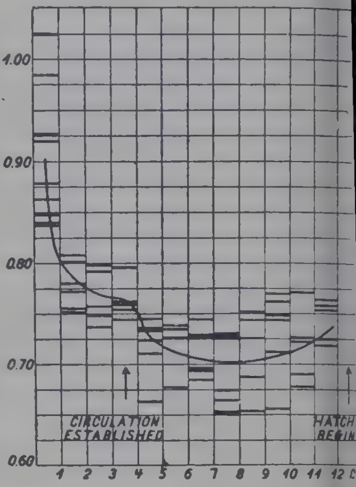

Fig. 313. Respiratory quoti during the development the minnow embryo (*Fun lus heteroclitus*).

The latter half of this process, at any rate, was made clearly visible in S. Smit work[1], where separate determinations of protein nitrogen and fat were made. T fat (% dry wt.) rises in the yolk (whether by synthesis or as a result of prot utilisation) from 10 at 0 days to 30 at the 80th day, remaining constant in t embryonic tissues themselves from the 30th day on. Total fat drops by 51 % the initial value (cf. Table 11). Protein drops also, by 49·3 % of the initial va (though 32·5 % is a better figure, as there may have been some starvation af exhaustion of yolk-sac reserves), but in absolute amount, this is a much grea ss than that of the fat. The noteworthy point is, however, that fat utilisation es not begin until the 56th day, and goes on till the 75th, while protein utilisa- on has already become considerable by the 40th.

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* We may leave out of account the oscillations in the curves of this author, to which un significance seems to be attributed by her.

† Krüger[2] has designed an apparatus especially for determining R.Q.'s of fish embryos.

Careful calorimetric estimations of heat production were also made throughout velopment. Like oxygen consumption, heat production rises regularly, from out 1 g. cal./100 eggs hr. to between 30 and 40. The full publication of the ta, with a comparison between heat found and heat expected, analogous to the ll-known Bohr-Hasselbalch balance-sheet for the chick (*CE*, p. 706), will be aited with interest.

Trifonova's work[3] on the development of the perch added some interesting ints to the above account. The greatest sensitivity of the embryos to anaerobiosis s found to be before segmentation had begun, and at this time also there were ximal amounts of lactic acid in the eggs. Lactic acid declined from an initial gh level to a final low one, suggesting an aerobic glycolysis at the earliest stages development (cf. below, p. 594). Yet intracellular pH, as judged by neutral d, becomes more acid (Trifonova[2]). Attempts to correlate respiratory rates with owth rates at different periods of development cannot, however, be regarded successful (Trifonova[3]; Privolniev[1]). Privolniev[2] claims to have found rhythms respiratory rate during early cleavages of the egg of the lamprey (*Lampetra viatilis*) — not a meroblastic egg — and these may well be justified, in view of e experiences of other authors, e.g. J. Brachet[4,7] (see below, p. 587), with phibian eggs.

Fish eggs differ considerably in their resistance to anaerobiosis. It was a classical servation of J. Loeb's that the minnow (*Fundulus*) is very resistant while the nner (*Ctenolabrus*) is not (confirmed for other eggs, e.g., by Trifonova & Popov ing various partial pressures of oxygen). Philips and Vernidub find that this is rrelated with their cyanide-sensitivity, though whether development proceeds not, the respiration falls by 80–90%. *Fundulus* eggs may therefore possess, frog eggs (see on, p. 588), the power of piling up an oxygen-debt, while se of other teleosts (cunner, scup, mackerel) do not.

mberson & Armstrong and Manery, Warbritton & Irving almost simul- eously discovered that an important alkali reserve is formed as teleostean eggs elop. In *Fundulus*, the first form investigated, this increase in bicarbonate ounts to 9 c.c. CO_2/100 gm. eggs in the 12 days of its development. It was ught that there must be a loss of chloride from the eggs to compensate for the v anion and the increase in buffering power, but when the phenomenon was firmed (by Irving & Manery[1]) on eggs of the trout *Savelinus fontinalis*, it was he same time found that there is a loss of chloride to the exterior far more than ancing the gain in bicarbonate, indeed seven times its size.* This unexpected balance is probably met partly by the new anion of inorganic phosphate arising m phosphatide and phosphoprotein breakdown, and partly by protein on the aline side of its isoelectric point. The subject may be further followed in the iew of Irving & Manery[2]. Retention of respiratory CO_2 for purposes of

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oss of chloride has also been reported for eggs of invertebrates, such as the gastropod *ifusus tuba*, studied by Kumon.

alkali reserve is probably a general phenomenon. It fits in with the mar bradyauxesis of chloride which we have already noted (p. 548, Table 30), with facts such as those collected by Surber on the optimal CO_2-content water in which fish eggs are developing.

We come lastly to a special case of much interest, that of the embryos syngnathid teleosts developing in the paternal brood-pouch, to which Leiner[3] devoted much study. He first found[1] that the respiration of the embryos of sea-horse, *Hippocampus brevirostris*, rose gradually to a maximum and then off, as follows:

	Q_{O_2}
Embryos from the brood-pouch:	
stage 1	−0·50
stage 2	−1·00
stages 4 and 5	−1·60
at "birth"	−2·84
Young fish some weeks old	−1·54
Adult fish	−0·66
Adult fish with embryos in brood-pouch	−0·85

The rise must represent the disappearance of inert yolk, and the fall the slowi of respiratory rate inherent in all tissues (cf. *CE*, p. 746 and below, p. 601). Leir also noted a distinct aerobic glycolysis in brood-pouch embryos ($Q_L^{O_2}$ + 0·53 the snake-fish *Nerophis ophidion* and + 1·71 for the sea-horse). But it was now c served[2] that *in vitro* in air the sea-horse embryos respired a good deal less intens than they did (by calculation of difference between "pregnant" and "non-pre nant" males) in the brood-pouch. Thinking that this might be associated with t fact that the Fe content of the brood-pouch liquid was 30–50 times as high as th of sea-water, Leiner tested the *in vitro* respiration of the embryos in the presen of different concentrations of iron, and found that in this way the respiration the embryos could be increased 100 %. The effect passes off in the later stag of development. Thus at stage 2*a* the increase may be 450 %, while at stage it may be only 20 %. The respiration of the embryos is very sensitive to C tension and osmotic pressure; the former naturally increases some four tim in the brood-pouch liquid during development, and the latter doubles. If the experiments should be confirmed they will establish one of the most remarkab known cases of dependence upon the parent organism, and show that althou we may have information in the case of an ovoviviparous animal whether t embryo gains or loses in organic substance during its development within t parental body, much of the interactions between the two generations may st be hidden from us. For in *Hippocampus*, as has already been mentioned (p. 4 the embryo is furnished with all the necessary organic substance by the matern ovary and takes none from the paternal body.

Another interesting adaptation occurs in the case of the Dipnoi. The eggs the lung-fish, *Lepidosiren paradoxa*, develop in burrows where the water contai no measurable dissolved oxygen (Carter & Beadle), but the male fish which guar the nest has long vascular filaments on its pelvic fins during the breeding seaso and J. T. Cunningham thought that these secreted oxygen into the water around t eggs. In order to test the matter, Cunningham & Reid[1] made a special journ the Amazon and carried out experiments in the field which showed that the gen-content of the water around a fish in such a condition can indeed rise. e emissive function was contested by Foxon, but the advantage of the argu- nt seems on balance to remain with Cunningham & Reid[2].

3·34. Respiration of the eggs of Amphibia

It has been unavoidably necessary to include much of the more interesting rk on the respiratory metabolism of amphibian embryos in Part 2 (pp. 193 ff.), it has so intimate a connection with the physiology of the organiser centre. re only those studies which concern the metabolism of the embryo and yolk a whole will be considered.

In the oviduct the amphibian egg discharges its large load of CO_2 (Dalcq, steels & Brachet, confirming older work), and not until a certain level has been ched does the egg become fertilisable. It seems fairly well established that re is no change in respiratory rate at fertilisation (J. Brachet[7]; Stefanelli[1]), and dence, not unconvincing, has been brought forward showing that there are lical variations of oxygen consumption during cleavage (J. Brachet[4,7]; Stefa- li[1]), but to ascertain exactly how they link up with the cytological changes will d further investigations (cf. p. 575). Should polyspermy occur, oxygen con- nption after fertilisation may be doubled (J. Brachet[7]).

As would be expected from the growth of the embryonic protoplasm at the ense of inert yolk, the oxygen consumption of the whole egg rises regularly m fertilisation until the end of the tadpole period (J. Brachet[6] on *Rana tem- aria*; Stefanelli[2] on the toads *Bufo vulgaris* and *viridis*; Atlas[2] on *Rana pipiens* l *sylvatica*; Wills on the urodeles *Triturus torosus* and *Amblystoma maculatum* l *tigrinum*). Following the respiratory rate until metamorphosis (see p. 453), lls observed in every case a decline in metabolic rate as soon as the yolk exhausted. This phenomenon is universal in growing organisms (see below, 01) and will be met with again in the discussion of birds and mammals. It is vn with isolated amphibian tissues such as skin (Börnstein & Klee).

he determination of respiratory quotient has been particularly interesting. careful study, J. Brachet found that it rose from a low level after fertilisation carbohydrate level at gastrulation:

Day	Stage	R.Q.
	Morula	0·66
	Advanced blastula	0·70
1	Gastrula	1·03
2	Neurula	0·98
5	Hatching tadpole	0·97

have already noted that these values were confirmed subsequently in the k on the R.Q. of the regions of the gastrula by Boell, Koch & Needham. That quotient in the post-hatching yolk-sac stage must be lower we know from the that some fat is combusted during that period. For example, Atlas[2] found about 250 c.mm. O_2 are required per individual of *R. pipiens* from fertilisation ne end of the yolk-sac period, during which time 0·2 mgm. dry weight are lost.

Were the whole of embryonic and pre-feeding larval life at the expense of car hydrate this figure would have been 149, or for protein 191 and for fat 400. first sight, therefore, the situation in the amphibia seems to contradict t generalisation that a succession of energy sources occurs during developme carbohydrate preceding protein and protein preceding fat.* But it may be t this succession only starts from gastrulation. In the examples given for f embryos (p. 584), no R.Q. for pre-gastrulation stages was available. And we ha seen some evidence in the more recent work on echinoderm eggs (p. 564) th there also pre-gastrulation R.Q.'s may be low. In view of facts now to be me tioned regarding the metabolism of amphibian cleavage stages, it may be suspect that low R.Q.'s of pre-gastrulation stages are due rather to special processes unusual type than to the catabolism of fat or protein.

In the first place it may now be regarded as certain that amphibian embry in the cleavage stages are exceptionally resistant to anaerobiosis. J. Brache found that under strictly anaerobic conditions cleavage and the formation of t blastula will proceed in the frog, though gastrulation is incomplete and neuru tion impossible. The toad *Discoglossus*, however, he found able to gastrulate a neurulate anaerobically. Spirito[5] later observed marked differences betwe amphibian species as to their power of withstanding anaerobiosis; toads such *Bufo* being in general able to develop further than frogs in the absence of oxyge Successful attainment of advanced tail-bud stages has even been reported, Lallemand. J. Brachet's lactic acid estimations[6] showed the existence of anaerobic glycolysis, rising with development, and later confirmed by Lenne strand and on isolated regions of gastrulae by Boell, Needham & Rogers. Und anaerobic conditions glycogen disappears (J. Brachet[6]). Brachet[6] was also able show experimentally that under these conditions the embryos accumulate oxygen debt, discharged by an excess oxygen consumption when they are broug out into air or oxygen. As the R.Q. of this excess oxygen consumption is very lo (0·3) oxygen must be used to build up an "oxygen reserve", which seem however, not to be in the form of oxidised cytochrome or glutathione. Anaer bically CO_2 is certainly produced (J. Brachet[6]; Latinik-Vetulani[2]).

J. Brachet's work[6] on the action of inhibitors revealed that respiration is mu more sensitive to KCN than is cleavage (a parallel with anaerobic conditions At $M/1000$, while segmentation is normal (though gastrulation and neurulati cannot proceed), respiration has been inhibited 90 %. At $M/5000$ even gastrul tion is possible. The converse effect is seen with phenyl-urethane. This narcot stops segmentation instantly at $M/1000$ but only reduces respiration 40 (a parallel with the work of Warburg on echinoderm eggs already referred t p. 517). Among other facts brought to light by Brachet was the slight aerob glycolysis shown by amphibian eggs, confirmed by Lennerstrand and (on isolat regions) by Needham, Rogers & Shen. Addition of dimethyl-*p*-phenylen diamine to the eggs showed a very active Warburg-Keilin system, and tests f the dehydrogenases showed them also to be active. Changes on fertilisation respect have not been found. Strong centrifuging does not affect the iration of gastrulae, but on crushing gastrulae to a brei there is an enormous, ugh transient, increase of respiration. This effect does not persist during elopment; on crushing tadpole tissues, the respiration falls by 50 %. All in the most striking speciality of amphibian embryos seems to be their "oxygen e" and resistance to anaerobiosis.

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* Hutchens has drawn attention to the fact that a similarly declining R.Q. curve is found in t cyclical development of *Chilomonas* cultures growing on acetate and inorganic salts.

n connection with this, it is interesting that Buchanan has found a special npensatory acceleration possible in amphibian development. Following osure to low temperatures, or, significantly, to KCN, in moderate concentra- ns, the treated embryos would develop more rapidly than the controls, ntually attaining equality with them. Thus acceleration of developmental may accompany the recovery from KCN inhibition of oxidations. Such eriments should be tried with anaerobiosis also. They are interesting in view he developmental accelerations and retardations which we have already seen he transplantation experiments of Harrison and his colleagues (p. 346). With 4-dinitrophenol no acceleration could be obtained (cf. p. 454).

emperature cannot be used to dissociate developmental rate from respiratory in amphibian embryos any more than it can in those of echinoderms (Atlas[1], allel with Tyler[5], p. 575). Amphibian embryo respiration was found by Duryee e sensitive to external osmotic pressure, being increased 62 % in Ringer and uced 21 % in distilled water. Slight acceleratory effects of radium emanation amphibian embryo respiration have been reported by Simon[2].

fter the appearance of haemoglobin (see p. 644) the circulatory system of the pole begins to play an important part in its respiratory exchanges. As the lt of a train of thought which began in the study of the respiration of mam- ian embryos (see p. 598), it was discovered that the haemoglobin present in al life is by no means the same as that of the adult or of the pregnant organism. e amphibia are the lowest group in which this fact has, up to the present, been ied. Working on the blood of the bullfrog *Rana catesbiana*, McCutcheon[2] was to show that the dissociation curves of the haemoglobin change markedly evelopment proceeds. Fig. 314, taken from his paper, illustrates this. The ociation curve, in the most general terms, moves from left to right and changes a rectangular hyperbola to a sigmoid curve, so that in the youngest tadpoles loading capacity is very high, while in the adult it is low. The two forms of noglobin (if there are only two) also differ in their reactions to *p*H. In sum, pears that the haemoglobin of the youngest stages is more adapted for con- ons of anoxaemia (presumably due here to relatively inefficient respiratory hanisms and to poor environmental oxygenation) than that of adults.

his ontogenetic change seems to be paralleled by certain phylogenetic changes, McCutcheon & Hall found that species of amphibia differ in the charac- stics of their haemoglobin dissociation curves, some approximating to the nbryonic type", others to that of the adult. In this there would be adaptive ificance (cf. below, p. 595). Similar adaptive displacements of the dissociation es of haemoglobin have also been found in fishes and correlated with their logy by Hall & McCutcheon. Thinking that the forms of haemoglobin in the developing amphibian might derive from different sites of origin (of w amphibia have three; spleen, kidney and bone-marrow), McCutcheon[2] splen mised bullfrogs, with the result that the dissociation curve of the blood Conversely that of spleen blood was unusually high. But it may be dou whether a given site of origin is limited to the making of one form of haemogl For the cytology of the blood cell generations, see Cameron[2].

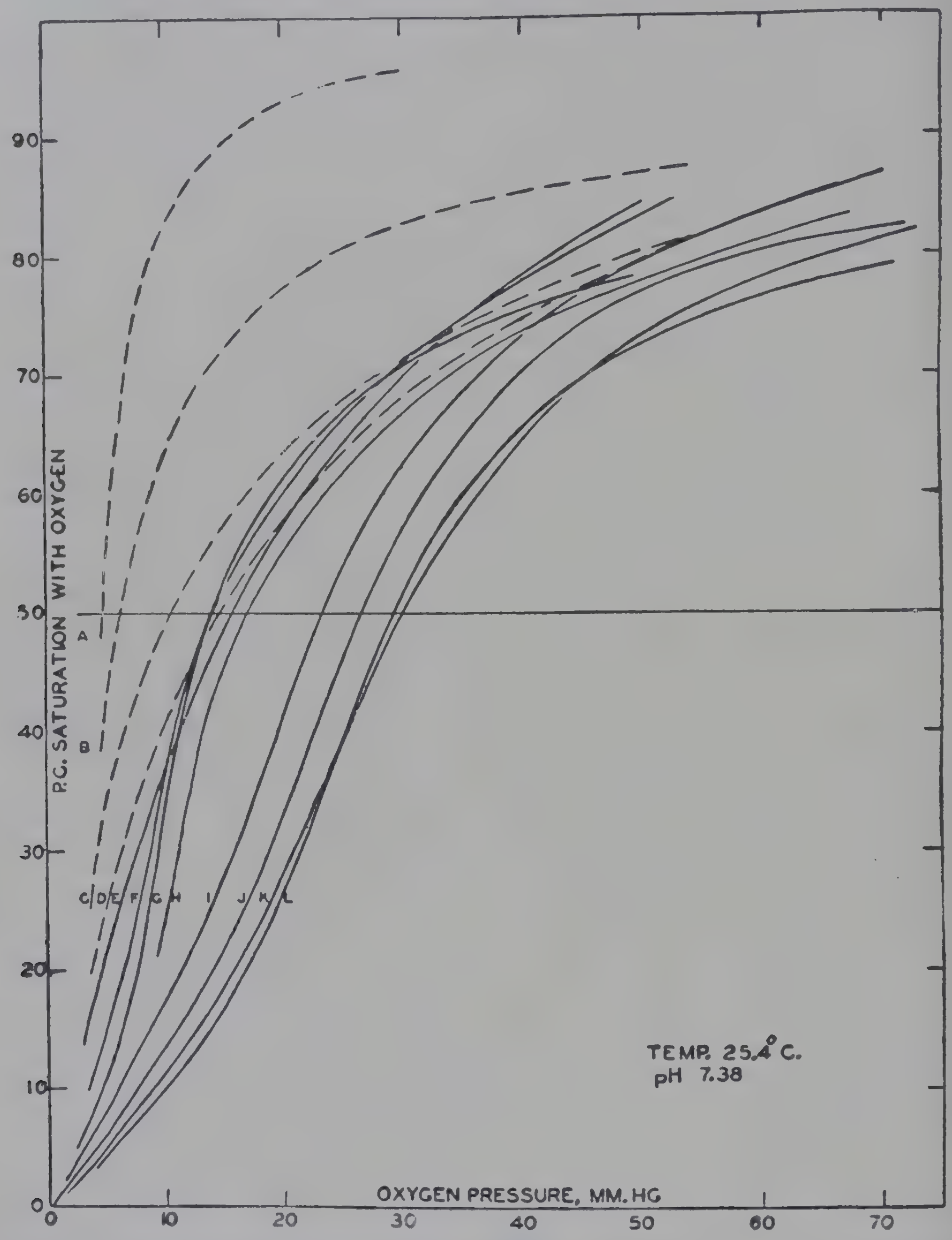
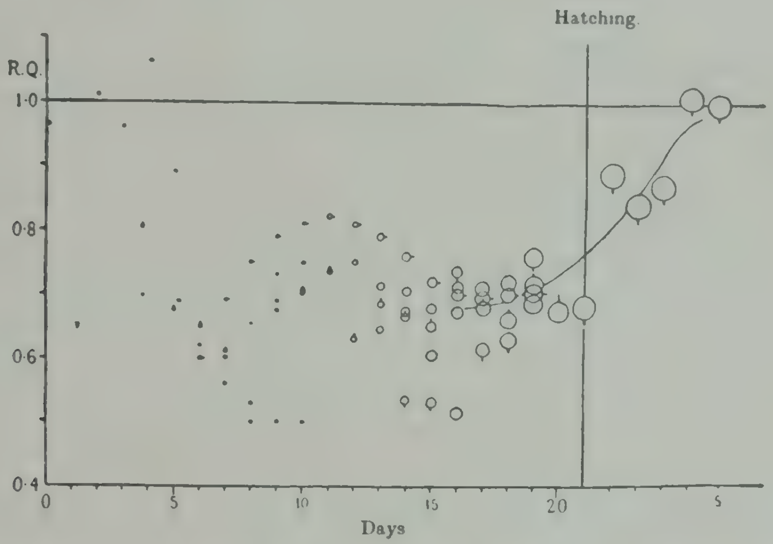


Fig. 314. Changes during the life history of the bullfrog in the dissociation curve of its hae globin. *A*, Tadpole 25 mm.; *B*, tadpole 92 mm.; *C*, legs well developed; *D*, respira method optional; *E*, tailstub still present; *F*, *G*, metamorphosis complete; *H–L*, adult.

3·35. Respiration of the eggs of Sauropsida

Extremely little attention has been paid to the embryonic respiratior reptiles, the only contribution being that of Zarrow & Pomerat on the egg smooth green snake *Liopeltis vernalis*. Towards the end of development R.Q. of 0·82 was obtained; after hatching it rose to 0·91—an effect parallel to seen with the chick (Fig. 315).

t is interesting that in later stages of the life cycle the metabolic rate (oxygen sumption per unit weight) of poikilothermic vertebrates steadily declines stner; Terroine & Delpech; Terroine, Hée, Roche & Roche; C. M. Liang). ce the surface law can hardly here be important, Kestner suggests that the line really signifies a decline in the proportion of actively metabolising protosm at the expense of "paraplasmatic" reserves and deposits. Senescence in t-developmental stages would thus be, in a sense, the converse of that process reby inert yolk gives place to respiring protoplasm during embryonic life p. 600).



315. Respiratory quotient during the development of the chick embryo (*Gallus domesticus*). The more accurate the determination the larger the circle. The sizes of the circles have been obtained by putting $d=w_1/w_2$ where d=diam. of circle, w_1=weight of living matter in the ystem, w_2=weight of inert matter including the shell. ♀ Lussana. o Bohr & Hasselbalch. ȯ Hasselbalch. o- Murray. (All on intact eggs.)

here is no new work on the respiratory exchange of the hen's egg during lopment, and that described in *CE* (p. 693) must be regarded as classical.* R.Q. was never easy to obtain owing to the large volume of inert material in ion to the respiring protoplasm, especially in the early stages. Nevertheless, tendency to obtain high quotients in the neighbourhood of unity during the week (see Fig. 315) has shown itself again in two new pieces of work along old lines (Hudiwara and Fukahori).

nvestigators have turned more and more, in view of these facts, to manometric hods, for which the chick embryo is suitable up to the 6th day of developnt. The extra-embryonic membranes are suitable, in view of their thinness uring diffusion, up to much later periods of incubation. By fixing isolated

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evertheless Noyons & de Hasselle have in progress a study of the heat production and CO_2 ut of the hen's egg throughout its development with new and extremely sensitive apparatus, h is said to distinguish between fertile and infertile eggs. They have already published a minary metabolic rate curve.

blastoderms of the first two days to silk discs with a plasma clot their R.Q. a could be obtained. As is shown in Fig. 316 (Needham[7]), the R.Q. of th blastoderms is mainly of a carbohydrate character, but if the *area op* alone is used, they fall between 0·7 and 0·8. From the 3rd to the 6th day onwa the R.Q. of the embryo is invariably 1·0, as was also found by Dickens & Šimer In the absence of added glucose, respiratory rate is very little affected, but R.Q. falls to about 0·85 (cf. the action of fluoride mentioned below). The R.Q.

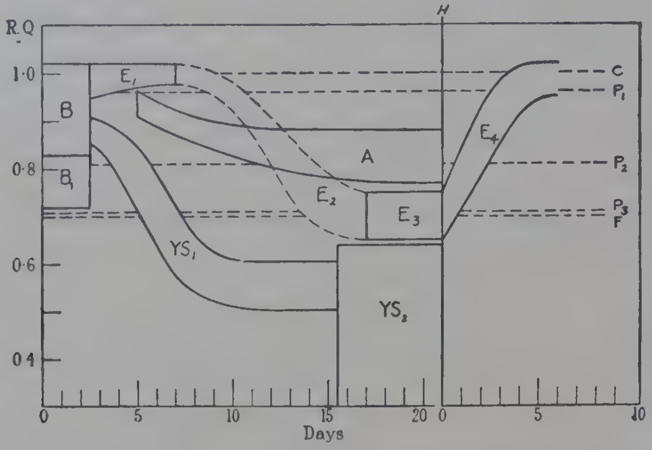

Fig. 316. Respiratory quotient of embryo and the separate embryonic membranes during development of the chick embryo. *H*, Hatching. Theoretical quotients: *C*, carbohydra P_1, protein, ending in ammonia; P_2, protein, ending in urea; P_3, protein, ending in uric ac *F*, fat; *B*, blastoderm, *area pellucida*; B_1, blastoderm, *area opaca*; E_1, embryo, manometri ally; E_2, embryo, assumed; E_3, embryo, from measurements on intact egg; E_4, chick, new hatched; *A*, allantois; YS_1, yolk-sac; YS_2, yolk-sac, late period.

the allantois falls slowly, but its values show that the catabolism of this importa membrane must always be mixed; here again the R.Q. is reduced in the absen of glucose. The R.Q. of the yolk-sac falls rapidly to very low levels, t meaning of which is not altogether clear. Working with a number of i hibitors, Needham[8] found that the respiration of the embryo is inhibited iodoacetate in moderate doses, but this is reversible by lactate; during su inhibition the R.Q. falls to 0·85. At the same dosage yolk-sac respiration is a inhibited, but irreversibly. Fluoride in doses sufficient to abolish anaerol glycolysis completely has no effect on the respiration of the embryo, which only affected by higher concentrations. But no matter how thorough the inhib tion, the R.Q. remains in the neighbourhood of unity, and this was quantitativ shown to be due to the occurrence of a protein catabolism ending in ammon (cf. the similar case of the grasshopper embryo, p. 581). Fluoride also inhib yolk-sac respiration, but at lower concentrations, and it has no effect on t yolk-sac R.Q. In general, it is clear that the embryo and the membranes duri early avian development have quite different metabolic qualities.* In particul the occurrence of protein breakdown after inhibition of carbohydrate breakdov by fluoride is interesting, because it shows that the preferential catabolism carbohydrate by the chick embryo during the first week of its development is r to any lack of the machinery necessary for catabolising protein. The same ng was demonstrated for ammonia production during respiration without strate, by Dickens & Greville[3] (NH_3 N γ/mgm. dry wt./hr. with glucose 0·1, h fructose 0·3, without substrate 1·0).

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* Kagiyama has studied the development of the Warburg-Keilin system in chick embryos b his results are obscure.

he metabolic rate of the chick embryo had previously been calculated, making allowance for the respiration of the membranes (*CE*, p. 707). Needham[6] was e to show that when recalculated on the basis of the *in vitro* oxygen consump- n of the membranes, it still falls, though only by about 500 c.mm./hr./gm. . wt. instead of 1000 as formerly thought. At the beginning of development 1 day) the respiration of the two principal membranes (allantois and yolk-sac) ounts for nearly 35 % of the total respiration of the egg; by the 19th day this fallen to 5 %. In this paper will be found the Plastic Efficiency Coefficient , p. 935) and the Apparent Energetic Efficiency (*CE*, p. 970) of the chick oryo, properly corrected in accordance with the new information on the abolism of the membranes. In the earliest stages of development the efficiency he yolk-sac is extraordinarily high, for only some 5 % of its material intake ombusted.

he fall of metabolic rate during embryonic life in the chick can also clearly seen in the Q_{O_2} of special tissues, e.g. liver (Carroll), lens (Kihara), retina yohara).* It is continued in the remainder of the life cycle, as indicated by nerous elaborate studies (Mitchell & Card; Riddle, Nussmann & Benedict; dy, Hall, Ragsdale, Trowbridge, Funk, Kempster, Ashworth, Hogan & cter; Brody, Procter & Ashworth; see Fig. 321).

omanov & Romanov[1] have studied the effects of varying concentrations of $_2$ in the air surrounding the eggs during ibation, and Remotti[6] has found that the ntoic blood-vessels can adapt themselves partial reduction of the shell area over ch gas exchange normally occurs. Romijn oos, taking up again the question of the pace (*CE*, p. 719), found that it increases a few tenths of a c.c. to 11 c.c. during nal development, while its O_2-content falls 20 vol. % to 12, and its CO_2-content from 1 vol. % to 6. There is work by Cunningham on the effects of increased en tension on development, but the lts are not striking.

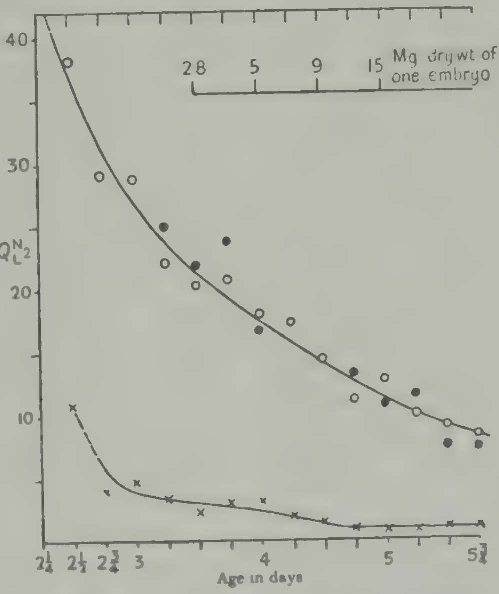

Fig. 317. Fall of $Q_L^{N_2}$ during development in the chick embryo. o Glucose; • mannose; × autoglycolysis.

s is well known, the anaerobic glycolytic of the chick embryo falls sharply during elopment, and this was found to be true Needham & Nowiński whether mannose glucose is used as substrate (Fig. 317). oglycolysis also falls slightly. The fall in $Q_L^{N_2}$ occurs early; at 2¼ days a value 35 is found, while at 5¾ days it is only +10. The Pasteur effect, i.e. the inhibition of fermentation by oxygen (discussed more fully in the succeedi section, p. 596), is marked in the embryo, whether glucose or mannose be t substrate. But the rate of oxidative disappearance of lactate is quite insufficie

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* The enormous $Q_L^{O_2}$ of the retina is said to arise only after hatching.

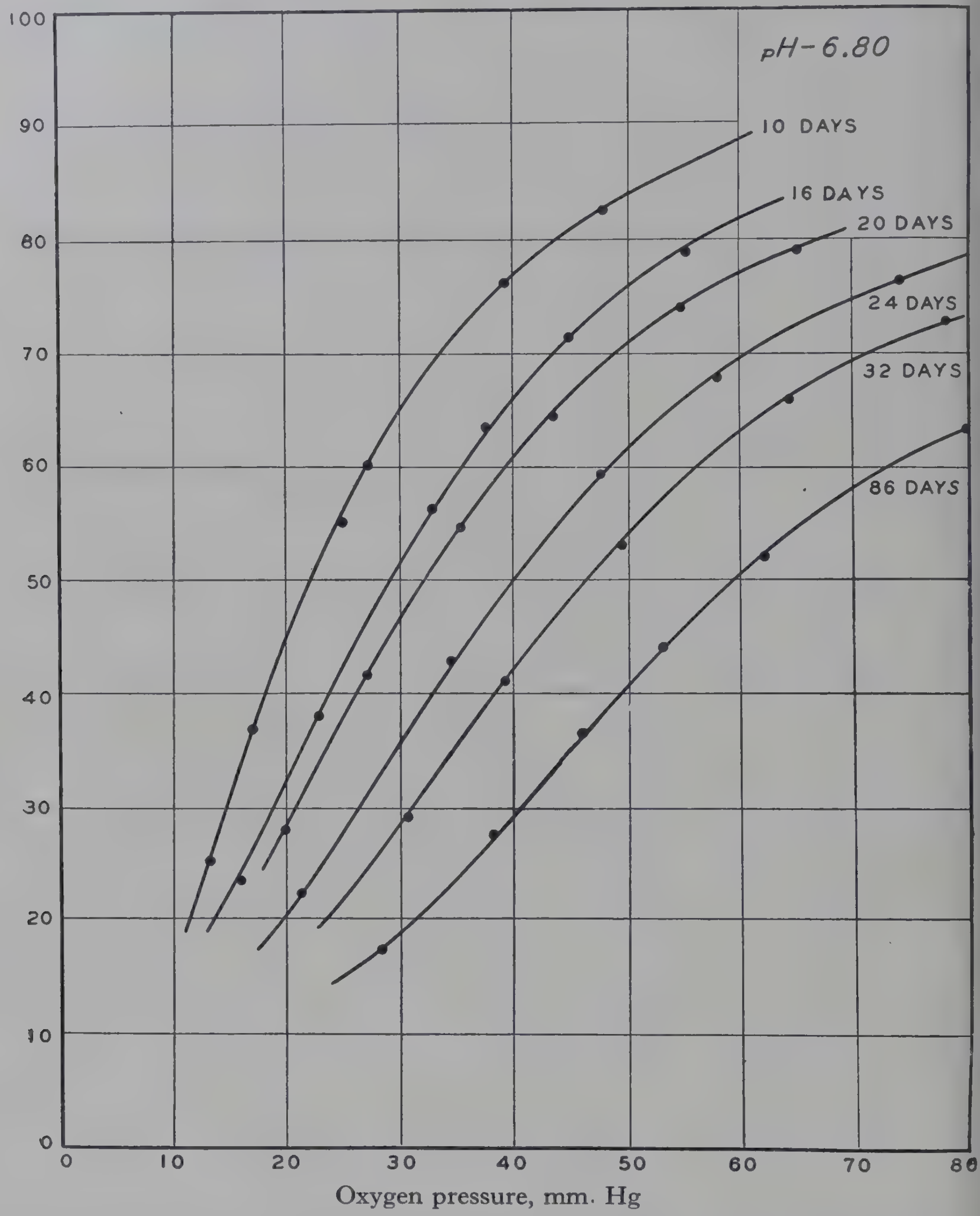

Fig. 318. Changes during the development of the chick embryo in the dissociation curve of haemoglobin.

to account for the effect of oxygen in reducing glycolysis (Needham, Nowiński Dixon & Cook), hence the Meyerhof resynthesis theory of the Pasteur eff cannot apply to the chick embryo.

It is usually said that the chick embryo shows no aerobic glycolysis. T statement may need modification in the light of some experiments by Lase from which it appears that the $Q_{L}^{O_2}$ of the chick embryo may be as high as +

2½ days' incubation, decreasing to nearly zero at 5 days and then (as tested on ces) rising again somewhat.*

Van Groor has studied the development and localisation of carbonic anhydrase ring the chick embryo's development. It appears in the blood at the 11th day d passes adult level on the 15th rising to nearly three times this on the 19th. e organ in which it is earliest detectable is the eye-cup, where there are preciable quantities as early as the 4th day; it is suggested that this is a pro- tive mechanism against the high aerobic glycolysis of the young retina.

Whether the haemoglobin of the chick embryo is identical with that of adult ds is an interesting question. The dissociation curve of chick-embryo haemo- bin was examined by F. G. Hall[2]. As shown in Fig. 318, there is the same trend the curves from the left to the right with increasing age, as was seen in the phibia (p. 590) and as we shall see in mammals (p. 599). Although the oxygen the embryo is obtained direct from the air, the shell is not easily permeable gases and the partial pressure of oxygen in the yolk and white is low. Thus en the oxygen tension in the environment of the embryo is low a haemoglobin present which has a greater affinity for oxygen, but after hatching, when quatic" respiration has given place to "aerial", this is replaced by a haemo- bin with less affinity for oxygen. As in the case of the amphibia these onto- etic changes may have phylogenetic parallels, for Hall, Dill & Barron have wn that the haemoglobins of certain birds native to high altitudes have a nificantly greater affinity for oxygen than those of other members of the same up of animals at sea level. As regards the mechanism of the change in the ck embryo, Dawson[2], in an interesting paper, gives tables showing the per- tage of various types of cell in the blood during incubation. He suggests that embryonic type of haemoglobin must be associated rather with the whole put of the yolk-sac's blood islands as opposed to the bone and marrow duction of later life, than with the specifically primitive blood-cells of the half of incubation which are not found at all during the second half.

·36. Respiration of mammalian embryos

nfortunately we have little or no information about the respiratory metabolism ammalian embryos in the earliest stages of their development — we know ing of the respiration of blastocysts, for example. Only the egg of the cow been investigated from this point of view, in the work of Dragoiu, Benetato preanu, mentioned elsewhere (p. 201) in another connection. Mammalian may, however, be excellent material for such studies and the full publication artesian diver studies on the rat is awaited with interest (see Boell & holas). Before implantation the respiration rapidly rises, presumably due tilisation of yolk or externally supplied nourishment. The $\underset{\sim}{Q}_{O_2}$ and $\underset{\sim}{Q}_L^{N_2}$ of e-slices of embryonic organs of rat and rabbit, falls off later with increasing

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curious feature of chick-embryo extract, noted by Frisch & Willheim, is that unlike extracts mour and adult tissue, it will not, when added to tissue the anaerobic glycolysis of which has stopped by reagents such as hydroquinone, restore the $Q_L^{N_2}$ to normal. But Pentimalli[3] s it accelerates the $Q_L^{N_2}$ of muscle. These effects are obscure.

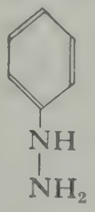
age, as would be expected, both before (Hanaoka) and after (Peruzzi; Pear P. D. Adams) birth. According to Kisch[1], they are more resistant to cert poisons such as $AlCl_3$ than adult tissues. But such tissue-slices, and also extra-embryonic membranes of rodents, have given a good deal of informat about the Pasteur effect and analogous problems in embryonic tissues, in the w of Dickens and his collaborators.

The Pasteur effect (to which a provocative review has been devoted by Bu is the name given to the almost universal suppression of fermentation by oxyg In most tissues, though not of course in tumours or in retina, glycolysis is redu to low or negligible values on passing from anaerobiosis to aerobiosis, wh respiration comes into play. On the Pfeffer-Pflüger theory this was because intermediate substances of fermentation are directly oxidised. On the Meyer theory (which, as we have just seen, does not apply to the chick embryo) fermentation intermediates are mainly resynthesised to carbohydrate at expense of energy derived from oxidations. On the Lipmann theory the int mediates are not formed at all in the presence of air, the enzymes of fermentat being themselves sensitive to the presence or absence of oxygen. The third these theories is to-day the most acceptable. It has received substantial supp from the finding of Schlayer and Kempner with bird red blood corpuscles t at oxygen concentrations intermediate between aerobic and anaerobic conditio the inhibition of respiration goes very far before the onset of fermentation begi The curves are not reciprocal and the rate of glycolysis depends therefore direc upon the oxygen concentration and not upon the rate of respiration. Las verified this in another way by finding that at low oxygen concentrations, respiration may be affected very little while the glycolysis is largely increas This was done on rat chorion and chick-embryo allantois as well as other tissu

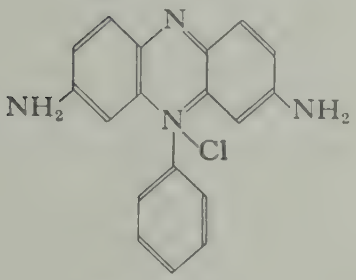
On the other hand, when in the presence of KCN respiration is decreas glycolysis will correspondingly rise, although the conditions may be perfec aerobic, as was shown long ago by Warburg on the chick embryo, and the sa is true of CO, as Laser[5] has shown. KCN thus not only inhibits the combinati of oxygen with the cytochrome oxidase, but also lifts the inhibition of t fermentation enzymes by oxygen. Hence it inhibits the Pasteur effect. Su inhibition has, however, been found to be brought about by many other su stances.

Dickens & Greville[2] worked with mammalian embryos (rodents and carnivor and their yolk-sacs and chorions. Like the chick embryo, such tissue has a la and prolonged autorespiration, indicating the presence of unknown intrin substrates. With added glucose its R.Q. is unity, without, it is about 0·85. Dicke & Greville[4] showed that unlike some adult tissues, the respiration of such tiss is not permanently affected by deprivation of substrate. As in the case of t chick embryo, urea formation is negligible, and ammonia is only produced wh no carbohydrate substrates are given. Again like the chick embryo (Needha Nowiński, Dixon & Cook), there is no stimulation of respiration and glycoly by K′ (Dickens & Greville[5]), though otherwise there are similarities with bra cortex (the high $Q_L^{N_2}$ and R.Q. of unity). Phenylhydrazine was now found kens[2] to inhibit the Pasteur effect in these embryonic tissues completely. So (as established in a later paper, Dickens[4]) does phenosafranin at only 10^{-5} *M* other substances related to pyridine, acridine, and quinoline, such as acri- in (Dickens).

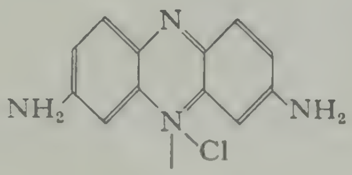
he mechanism of these effects is still not fully elucidated, but while it seems ly that KCN (Warburg), CO (Laser), 1-amino : 2-naphthol : 6-sulphonic acid ah) or glutathione (Bumm & Appel) all act by uniting with iron, it is suggested



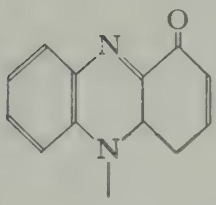
Phenylhydrazine



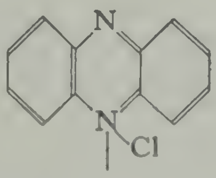
Phenosafranine



Acriflavine



Pyocyanin



Phenazine methochloride

substances containing heterocyclic nitrogen in six-membered rings and so nbling the co-enzymes (cf. p. 209) act by displacing them on their protein ers. But some of these, such as pyocyanin and phenazine methochloride, h, as we have seen (p. 568), accelerate respiration, increase the Pasteur t by abolishing aerobic glycolysis altogether (Dickens[3]).

urning to the processes of oxidation, we find that there are indications of a ial position occupied there also by embryonic tissues. The Szent-Györgyi- os cycles of C_4 compounds are now recognised as playing an important in oxidations (cf. Fig. 307). It is of interest, therefore, that Blazsó states embryo rat tissues will not attack either fumarate or oxaloacetate, and that rding to Elliott & Greig the succinoxidase activity of the chick embryo is slight. So is that of the echinoderm embryo (Krahl, Keltch, Neubeck & ves). Greig, Munro & Eliott indeed consider that the succinate-fumarate- ate-oxaloacetate cycle cannot occur in the chick embryo, because none of e substances are attacked by it.

The question of anaerobiosis and embryonic life provides a suitable transit here. It has long been suspected that embryonic cells are more resistant anaerobiosis than those of adult animals (see *CE*, p. 742 and above, p. 52 This has been studied mainly on tissue cultures, but there is some difference opinion as to what these can do, Laser[2] maintaining that chick embryo he fibroblasts can grow anaerobically (provided they have been first allowed aerobic period), though this is contested by Havard & Kendal. At the end embryonic development in the rat and mouse the foetuses are remarkably sistant to asphyxia (M. Reiss), and a quantitative study was made of t phenomenon by Enzmann & Pincus. In spinal mice the interval betwe decapitation and the disappearance of all spinal reflexes decreases enormou (from 1200 to 30 sec.) during the first fortnight after birth. If, then, embryon cells are more resistant to anaerobiosis than they later become, it is of inter not merely because many have thought, as did Pütter in 1905, that anaerobio might be regarded as more "primitive" than aerobiosis, but also because the is reason for thinking that towards the end of foetal life, in mammals, conditi of partial physiological anaerobiosis may be encountered.

The clearing up of our ideas on this subject is due mainly to the extensi work of Barcroft and his collaborators, reviewed in several places* such as t monograph of Windle). As we have already seen (p. 79), the placenta do not grow at the same rate as the foetus, but attains its maximum size and blo volume early. But the volume of blood passing through the foetal heart an the oxygen consumption of the foetus continually increase, and this is com pensated for by increasing blood pressure during development. In the shee for example, the blood pressure of the foetal circulation rises from 20 mm. H at 50 days to 76 mm. Hg at birth.† But while the foetus grows and the blo flow through the foetal part of the placenta increases, conditions on the matern side remain comparatively steady. Hence there should be a continually i creasing deoxygenation of the maternal blood passing through the placen and this has been shown experimentally to be the case (Barcroft, Herkel & Hi Barcroft, Flexner, Herkel, McCarthy & McClurkin). In the rabbit the pe centage saturation of the uterine vein blood falls from 85 to about 15 at the e of pregnancy. The oxygen capacity of the foetal blood rises, but its percenta saturation remains at about 45 (Barcroft, Kramer & Millikan).

It may now be taken as established that diffusion processes alone account f the passage of the blood gases through the placenta (Haselhorst & Stromberger[1] Keys[2]). The more thoroughly, therefore, the maternal haemoglobin gives up oxygen at the pressure existing on the maternal side, and the more thorough the foetal haemoglobin accepts it at the pressure existing on the foetal side, t re efficient the transfer will be. Apart from the relative amounts of haemobin in maternal and foetal bloods, the important factor here will be their pective dissociation curves, and it was when these were studied that the most eresting physiological adaptations were brought to light. Fig. 319, taken from croft[5], shows such curves. The steeper the curve in the middle portion and further it is to the right, the more readily will the blood yield up oxygen; less inflected the curve and the further it is to the left, the more readily will blood take up oxygen. The foetal blood should therefore be to the left, maternal to the right. This is exactly what was found. Fig. 319 shows average curves for the goat, and the dotted lines give the normal limits variation in adults (Barcroft, Elliott, Flexner, Hall, Herkel, McCarthy, Clurkin & Talaat). This divergence of foetal and maternal bloods probably urs in all mammals; it certainly occurs in man* (Eastman, Geiling & de

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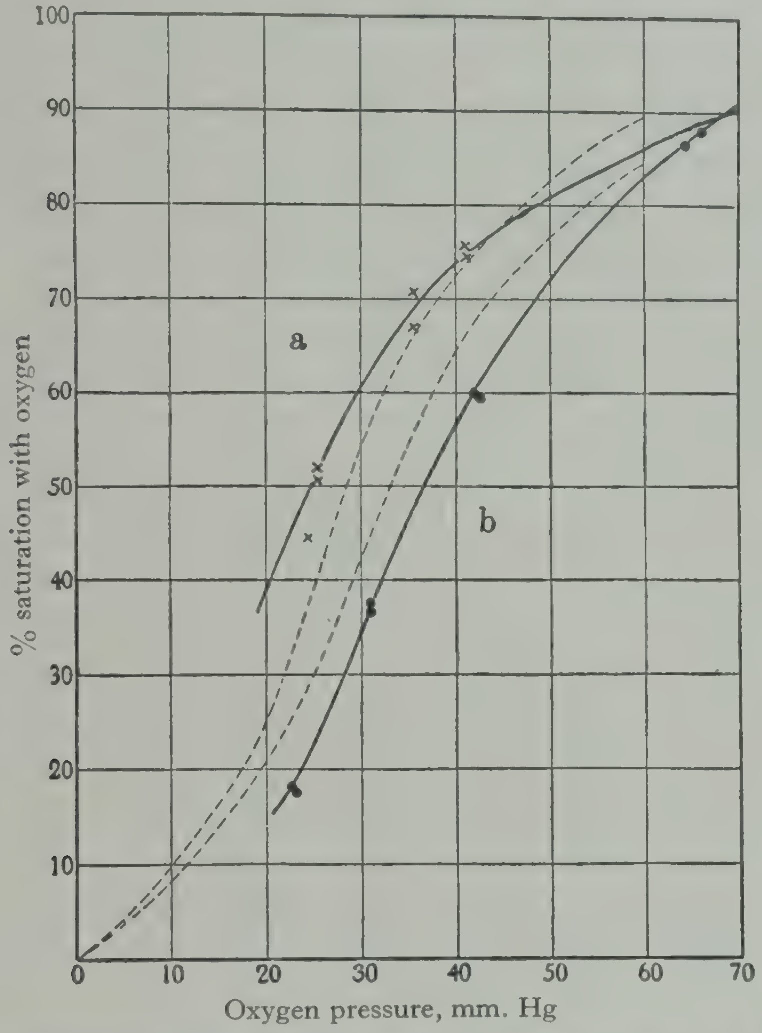
* Barcroft[3,4,5,6,7]. Here we omit consideration of the genesis of the respiratory movemen with which this subject is connected (see Barcroft & Barron; Barcroft, Barron & Matthe Barcroft, Barron & Windle; Steele & Windle; Windle, Monnier & Steele; Snyder & Rosenf for mammals; Windle & Nelson; Windle, Scharpenberg & Steele; Windle & Barcroft; Z. Y. Ku Z. Y. Kuo & T. C. Shen for birds). N.B. also the interesting problem of the closing of the duct arteriosus (Barclay, Barcroft, Barron & Franklin; Barclay & Franklin).

† Blood-pressure reflexes of mammals do not develop till after birth (G. A. Clark[2]).



319. Typical dissociation curves of (*a*) foetal, (*b*) maternal, blood, in a mammal. Dotted lines indicate limits of normality.

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his was for a time uncertain because of the work of Haurowitz, but it has been shown that an haemoglobin is very sensitive to dilution and that when the conditions are adjusted, the rence in the dissociation curves can readily be seen (Hill & Wolvekamp; Likhnitzkaia &

Lawder; Sax & Likhnitzkaia; Leibson, Likhnitzkaia & Sax), in the cow (R & Romijn) and in rodents. It would be of the greatest interest to find wheth it occurs also in non-mammalian viviparity and ovoviviparity. These were t findings which stimulated the search for similar displacements in foetal for of haemoglobin, the results of which have already been mentioned under t headings of amphibia and birds. But in evolution the adaptation to embryo anoxaemia must have preceded viviparity and hence also the correspondi compensatory maternal shift.

The mechanism of these dissociation-curve differences has provoked mu research. On the maternal side it is sure that the effect is due to an increase blood acidity (Hoag & Kiser). But on the foetal side it has been established t a special form of haemoglobin (presumably differing in its protein moiety) present (F. G. Hall[1], manometrically; McCarthy[1], spectroscopically). Ma interesting points arise in connection with the foetal form of haemoglobin. may be noted that in higher affinity for oxygen it approximates more to mus haemoglobin, studied by R. Hill[2,3], which functions at pressures intermedi between that of the active cytochrome oxidase and the venous blood, just ordinary haemoglobin functions at pressures intermediate between those arterial and venous blood. Moreover, the ontogenetic change is paralleled by phylogenetic one, since Hall, Dill & Barron have found that the haemoglobi of mammals living at high altitudes have a greater affinity for oxygen than tho of similar animals living at sea level. Indeed, the parallel between life at hi altitudes and the situation of the foetus at term had not escaped Barcroft. the top of Mount Everest, he wrote, the partial pressure of oxygen in the alveol air would be about 30 mm. Hg, and it seems probable that an oxygen pressu of 24 mm. (which is that of the blood going to the goat foetus towards the e of pregnancy; Barcroft & Mason) would be incompatible with consciousness man. Hence the phrase "Everest in Utero" (Barcroft[4]).

Nothing can yet be said with certainty about the origin or the disappearan of the foetal form of haemoglobin. But it has been shown that in adult mamm there is more than one form of haemoglobin (Brinkman, Wildschut & Witte mans; Brinkman & Jonxis), though these cannot be distinguished spectrophot metrically (Jongbloed). A good deal is known, as can be seen from the accour of C. Smith and Enzmann[2], about the blood picture during development mammals, but so far it has not proved possible to correlate the different for of haemoglobin with different morphologically distinguishable types of erythr cyte or with different sites of production. There is, however, a change in the about the time of birth, whereby the large foetal corpuscles (diam. 9·17 μ) a replaced by small adult ones (diam. 5·88 μ), see on, p. 648.

3·365. The rise and fall of metabolic rate

We may find it convenient to regard the metabolic rate of late pre-natal li as one of adaptively depressed metabolic rate. It is sure that in mamm (Barcroft, Kennedy & Mason; Barcroft, Flexner & McClurkin) the total oxyg consumption of the embryo is four or five times less than it is shortly after birt e are probably right in viewing this as connected with the respiratory diffi-lties occurring towards the end of gestation, but the phenomenon is seen in rds as clearly as in mammals. Fig. 320, taken from the paper of Brody, Hall, ıgsdale, Trowbridge, Funk, Kempster, Ashworth, Hogan & Procter, shows e minor peak in metabolic rate after hatching in the chick.* The major peak metabolic rate in the life cycle exists at a much earlier stage; in the chick rtainly before the 6th day of incubation (Needham[6]) and probably before the ıd (Philips). We have seen it in amphibia (Wills), fishes (Leiner) and many her animals. In embryos with holoblastic cleavage, the peak will appear to be

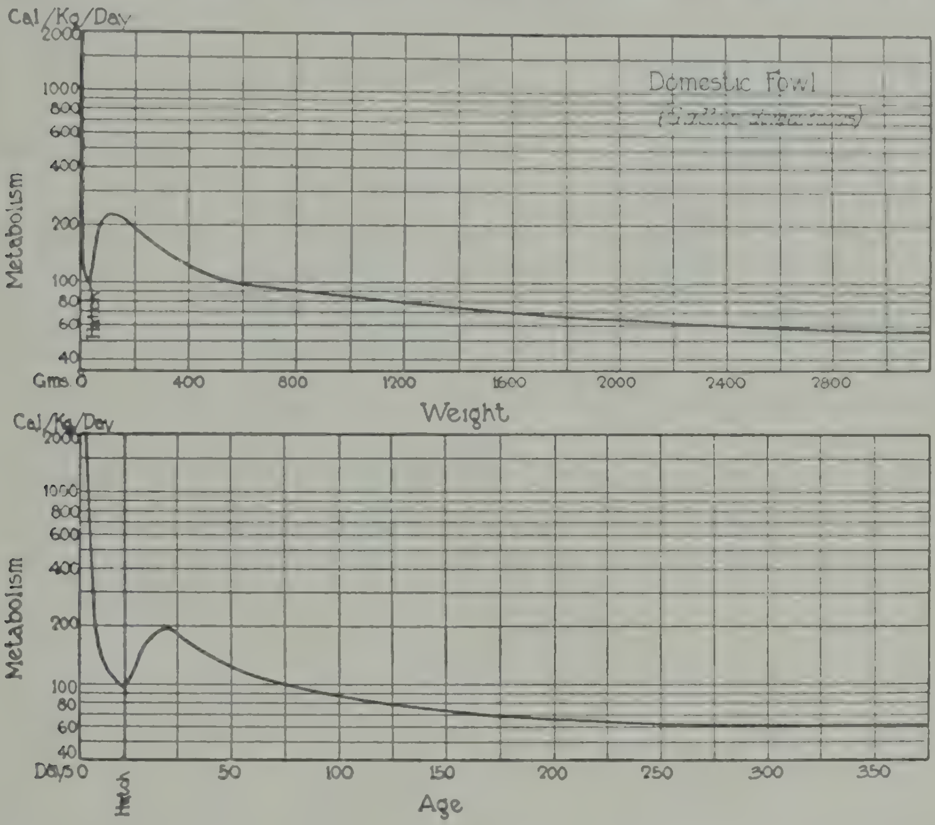

Fig. 320. Metabolic rate during the life-history of the fowl; above, related to weight; below, to age.

fted to a relatively later stage in the life cycle than in embryos with roblastic cleavage, for the cells of the former are more loaded with yolk utilisation of which is a pre-requisite for the manifestation of the peak l the downward trend. The universality of this downward trend includes only the homoiothermic organisms usefully brought together by Brody *et al.* Fig. 321, but also poikilothermic organisms, where the loss of heat by radiation m the steadily decreasing surface area does not apply. It is best to regard the l of metabolic rate as due to the same cause as its rise during embryonic velopment, namely the relative proportion of respiring protoplasm and inert ıterial in the cells. At the same time, the surface factor must not be excluded. s still required to explain the difference between large and small homoiotherms

solated tissues show this as well as the intact organism, as witness Carroll's figures for liver ; chick embryo 6th day −7·53, 20th day −1·53, after hatching −2·35, two years old −1·42.

at equivalent stages in the life cycle (cf. H. Blank). These views do not disa with the speculations about the rise and fall of metabolic rate which have b made by Jordan-Lloyd and Dhar.

The difficulties of determining the metabolic rate of the chick embryo ar course far greater in the case of mammals, especially man. Apart from

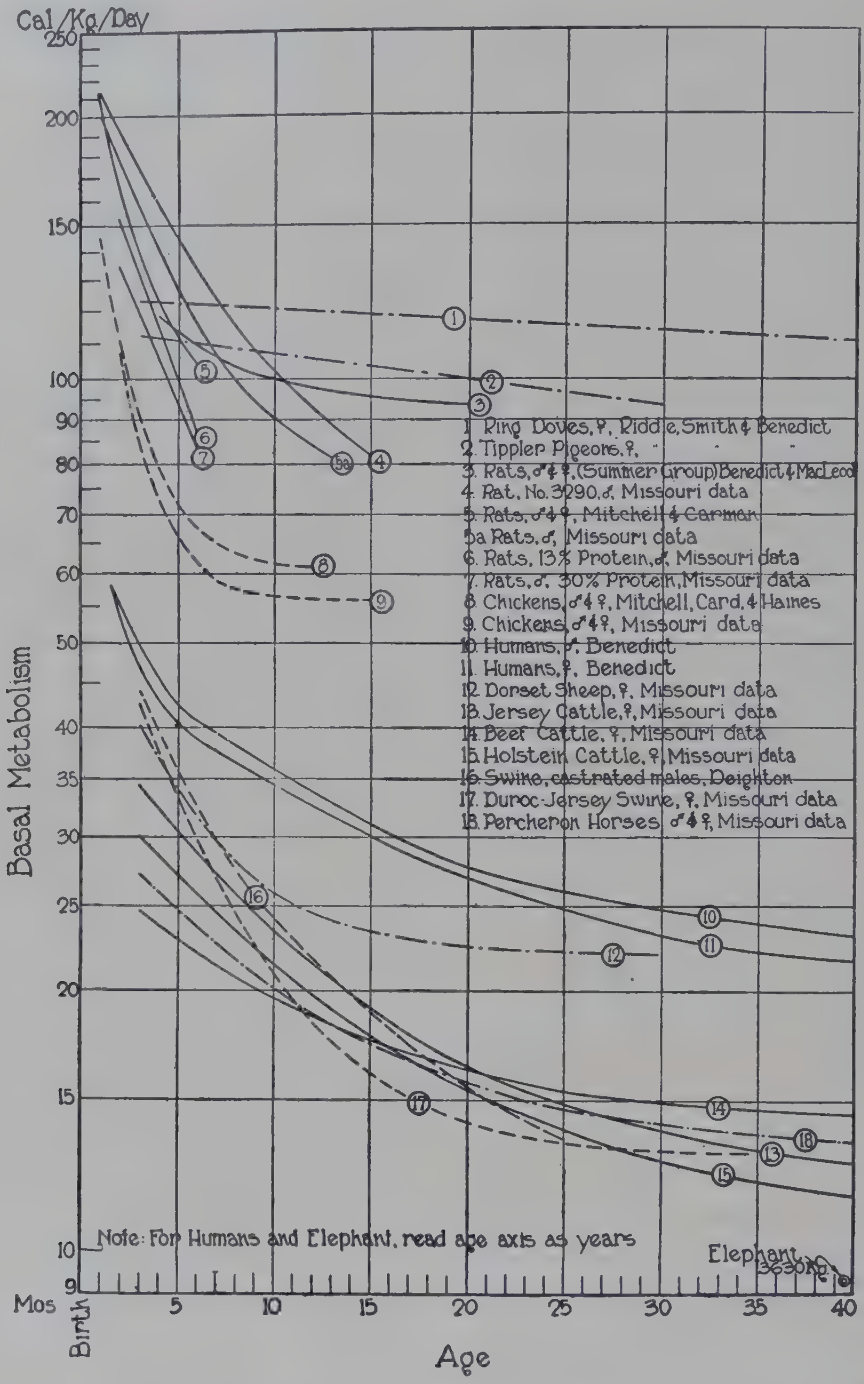

Fig. 321. Metabolic rate during the life-history of a number of birds and mammals.

gasometric determinations of Barcroft and his collaborators, just referred there are numerous studies with calorimeters on man (Plass & Yoakam; Schw & Drabkin; Rowe & Boyd), but the contributions of foetus and mother peculiarly difficult to disentangle, and cannot be discussed here. On a revi he data for all mammals, it has been concluded that the production of 1 kg. h weight of foetus is associated with a gestation heat increment of about 0 cal. and that the magnitude of this increment varies with approximately the power of the offspring's birth weight (Brody[2]; Brody, Riggs, Kaufman & ring).

astly, the subject of the genesis of body temperature regulation in homoiotherms iewed since *CE*, p. 772, by Deighton) may be referred to. There is no doubt in nidicolous mammals (cf. p. 76) there is a remarkably rapid onset of power of temperature regulation (Pincus, Sterne & Enzmann; Stier; Gulick; oschkina). The details must be sought in the original papers.

·37. The succession of energy-sources

early twenty years ago it was remarked (see *CE*, p. 986) that embryonic elopment seemed to show a succession of energy-sources, carbohydrate eding protein and protein preceding fat. If we glance through the foregoing s we see that this is now established for many organisms, the chick (p. 592); h, the minnow (p. 584); an insect, the grasshopper (p. 580); a crab (p. 577); matode (p. 576); and a gephyrean worm (p. 575). Where it has been possible, ever, to measure the respiratory quotient in the cleavage stages, values more acteristic of protein or fat have been found, e.g. amphibia (p. 587) and sea-ins (p. 564). If we may assume, though it is by no means proved, that these tients are to be interpreted in a normal way, we reach the coherent hypothesis gastrulation is accompanied by carbohydrate catabolism, and that the ession begins, as it were, from that point onwards. The only observations mpatible with this view are those of Horowitz[3] on the gephyrean worm, *chis*, in which the fall of R.Q. commences from fertilisation.

·38. Respiratory enzymes

the course of the preceding sections of this discussion there have been a many references to the enzymes concerned in respiration. The purpose of resent section is to add a few points which have escaped mention up to now. iere has never been any systematic description of the dehydrogenases and quantitative activity during embryonic development,* but it is to be ned that these arise *pari passu* with the respiring protoplasm. They are inly weak in some embryonic tissues (Banga and Blazsó for pyruvic dehydro-se; Breusch for fumaric, succinic and oxaloacetic dehydrogenases; Glezina ıccinic dehydrogenase; see also pp. 612, 615). By the 8th day of incubation, nic, lactic, hexosediphosphate, α-glycerophosphate, and glucose, dehydro-ses are all present in the chick embryo (Booth), but neither xanthine oxidase the Schardinger aldehyde oxidase appear till a day or two later (evidence for identity), though, as E. J. Morgan found, they are both in the yolk-sac al-y on the 7th. In embryo extract, prepared from 8-day embryos, as for explant a, Sachs, Ephrussi & Rapkine found xanthine oxidase, succinic, lactic and dehydrogenases, and glucose dehydrogenase.

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* The paper of Simola, Stenberg & Uutela I have found inaccessible.

It is unfortunate that more attention has been paid to the less importa auxiliary enzymes, catalase and peroxidase. The function of catalase is n generally allowed to be the elimination of hydrogen peroxide arising as the produ of the primary dehydrogenase reactions. Catalase accomplishes this by addi oxygen to the peroxide; peroxidase does the same job, but by adding hydrog and simultaneously oxidising certain aromatic compounds, either intrinsic artificially added, such as benzidine or guaiacol.

Contrary to a persistent belief, it is not possible to show any relation betwe catalase activity and respiratory intensity.* This is illustrated by work on insect of which there is a good deal (on the silkworm, Yamafuji; Yamafuji & Got on the mealworm, Charikova & Mikhailov; on the gipsymoth, Kozhanchikov The best study is that by M. E. Williams on the grasshopper, *Melanopl differentialis*. Catalase shows minimal activity till the 8th day of developmen the curve then climbs during the pre-diapause respiration peak and reaches constant level, which it maintains during the earlier part of diapause. At t beginning of post-diapause, however, it is found to be at a lower level, aft which it rises steadily until hatching. Or again, in the eggs of amphibia such the toad *Bufo*, Friggeri has shown that there is no change in the amount catalase until the external gills appear; it then increases rapidly to the end of t yolk-sac period, when the original activity is about doubled.

Both catalase and benzidine peroxidase (cf. pp. 136 ff.) are widely distribute in the eggs of echinoderms, fishes and amphibia, mostly at the animal pole of t latter, according to Sammartino and Spirito[2,3,4], and evince no change at ferti sation. In the echinoderm egg catalase, like dipeptidase, is associated with t hyaline ground-substance (Holter[2]; Holter & Linderstrom-Lang) but the dehydr genases are, to a certain extent, tied up with the centrifugable granular elemen (Ballentine[3]). The chick embryo shows benzidine peroxidase in the blastoder especially the yolk islands, well before the appearance of any haemoglobin.

For the embryos of birds and mammals there are good quantitative studie showing that the activity of catalase per unit wet weight increases with a (e.g. Kleinzeller & Werner and Kolobkova for the whole chick embryo; Galvia & Goryukhina for chick embryo blood; Vadova for the rabbit embryo). T former curve rises as if it were the reciprocal of the declining glycolytic ra (*CE*, Fig. 175). On the other hand, in the last half of incubation there seems be a fall of activity (Schkljar[2,5], who related activity to dry weight). Wh Blagoveschensky compared the temperature coefficients of catalase from anima of different ages, he found marked and progressive differences. This is interestin for it would mean that there are changes in the protein component of the enzym with age, analogous to those we have already seen so well established for t haemoglobins. Vadova subsequently found that the catalase of rabbit tissu changes in this respect just about the time of birth, and Kolobkova reports continuous fall in its temperature coefficient during the development of the chi embryo.

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* There is, however, the remarkable fact observed by Yamafuji, that the optimal conditions f breaking the diapause of silkworm eggs (exposure to 20 % HCl for 4 min. at 50° C. on the 12 day) are also those for maximal catalase activity.

·39. Axial gradients and respiration

: may be advisable to conclude this section by a word concerning the axial lient work carried out by the school of C. M. Child. This was recently ımarised in two reviews by him, and we now also have the valuable biblio- hy of his papers prepared by Hyman & van Cleave. The standpoint formerly n (*CE*, pp. 582 ff.) was that although gradients of susceptibility to lethal its, intensity of vital staining, etc. had been clearly demonstrated in a large ıber of adult invertebrates and many embryos, no evidence whatever had n brought forward to justify a belief in the existence of "respiratory" or etabolic"* gradients in embryos, and very little satisfactory evidence for this ıe case of adult organisms. I see no reason now to modify these conclusions. ser analysis strongly indicates an identity of respiratory rate in the organiser on and ventral ectoderm of the amphibian gastrula—a test case for the spiratory gradient"; cf. p. 199. As regards adult organisms, results have been e contradictory. Reference may be made to the (somewhat unconvincing) y to various critics by Watanabe & Child and the subsequent renewal of the ck by Maluf[2].

ut when all this has been said, it is sure that embryology owes a considerable t to Child, who by introducing his gradient concept was one of the first to gnise those invisible dispositions of order with which the organism imposes rganisation on the matter of which it is made. To-day the gradient theory merged in the general theory of morphogenetic fields, already discussed . 127 ff.). It is not likely we shall be able to do without this in the future gress of embryology.

. Metabolism

n this section we return at last to what was the main subject discussed in *nical Embryology*, namely the chemical changes which go on side by side the process of morphogenesis. But before proceeding to the various special s of metabolism in their order, there are a few points of a general kind which receive brief mention. Besides those investigations which have approached iochemistry of the embryo directly, there are a number of indirect ways of k which throw more or less light on it.

ne American biologists have been unusually vague in the meanings they have attached to the "metabolism". Whereas the school of Child regarded the time taken for a piece of a worm to lve in KCN solutions, or the intensity of staining with a vital dye, as throwing some light ıetabolic differences, certain European writers seem to think as if metabolism were purely ıtive respiration, purely a matter of "fuel" rather than "machine". It is needless to say that e present book "metabolism" is given its classical definition as the sum total of all the chemical ges going on in the body; it therefore includes the structural alterations of the proteins which he basis of all morphology. Modern work with isotopes has demonstrated how the proteins tantly participate in metabolism. The term "metabolic rate", as used by the calorimetric ıologists, refers specifically to heat production in calories, always related to the wet weight, veight, or surface area of the organism. The school of Child cannot but plead guilty to the ;e of having had a concept of metabolism too wide and vague; others, however, have thought in an unduly narrow way.

3·41. Indirect approaches

Changes in density of the egg system during development are an example this. The well-known decrease in density of teleostean eggs during their develo ment (*CE*, p. 822) probably occurs as a result of fat utilisation. Amphibian eg as we know from an admirable paper by Briggs[1], also decrease in density duri development, from fertilisation to the advanced tadpole stages, though the chan is interrupted during the time of closure of the neural folds, when there is transient density increase. There can be no doubt that the main trend here due to the absorption of water by a non-cleidoic egg (cf. p. 35) and the transie density increase to the occlusion of the archenteron which amounts to a tempora "solidification" of the system. In molluscan eggs also there are interesti density changes (Comandon & de Fonbrune[1,3]).

Another way in which it has been hoped to gain information about the gene course of events in embryonic life is the measurement of the osmotic pressur According to the description of Backman & Runnström (*CE*, p. 778) which h become classical, the osmotic pressure of the amphibian egg sinks shortly aft fertilisation from Δ – 0·48° to about the level of the fresh-water medium, apparent owing to a binding of nearly 90 % of the osmotically active substances. Th account can no longer be accepted, for the work of Krogh, Schmidt-Nielsen Zeuthen has shown that the fall is only a slight one (20 %); rapid till the fir cleavage, slower from then till the beginning of gastrulation. After gastrulatio a rise sets in until the original value is approximately regained at the time maximal development of the external gills. The early fall must be interpreted again due to the absorption of water by the whole system (see p. 38, Table 6 though there are indications of a slight loss of Cl′ ions; the later rise must be du to the production of small organic molecules which cannot escape owing to th very low permeability. During later development there must be many change in the osmotic pressure of the internal medium, as is shown, for example, in th work of Fontaine & Boucher on eels.

Osmotic pressure measurements, of more or less dependability, are als available for the amniotic and allantoic liquids of birds (Howard[3]), chelonia reptiles (Imamura[1]), etc., but their significance is minor. A careful study of th electrical conductivity, surface tension, and *p*H of the chick's amniotic and allantoic fluids was made by P. A. Walker. In the latter the conductivity falls unt the 8th day, is stable till the 15th, and falls thereafter catastrophically, as would b expected from what we know of its ionic composition. The surface tension fal until the 13th day and then rises again to its original value, a fact more difficult t explain. The *p*H is roughly stable at 7·4 till the 13th day after which it fal steadily to 5·6 (see p. 622). In the amniotic liquid the conductivity is high unt the entry of albumen through the sero-amniotic connection, when it rapidly falls Surface tension tends to rise throughout development except after the 16th day and during this same late period, the *p*H, which earlier has been constant aroun neutrality or slightly falling, rises to 7·8.

After earlier controversies (described in *CE*, pp. 839) it is now generall accepted that the *p*H of the cell interior, and of egg-cells in particular, is in th close neighbourhood of neutrality (e.g. the value of 6·8 given for the egg of th sea-urchin by Pandit & Chambers and for that of *Fundulus* by Chambers[2]). Bu where the meroblastic egg has become a large and complex structure, as in birds the neutrality characteristic of protoplasm is departed from. Thus all observer find the egg-white of fowls' eggs to be as alkaline as *p*H 9·0 and the yolk betwee 5·0 and 6·0 (Gaggermeier; Moran[4]; Penionskevitch[2]; Berenstein & Penionskevitch y also all agree that as development proceeds yolk and white attain neutrality even overpass it somewhat in the opposite directions. The alkali reserve eases in the albumen and decreases in the yolk (Schkljar[3,4]; Rubinstein) and titratable acid in the amniotic liquid rises (Remotti[5]). Other terrestrial eggs, h as that of the silkworm moth, also show variations in pH during development garo[1]; Tirelli[5]).

ince the former discussion (*CE*, pp. 865 ff.) of *r*H, little that is relevant here been done on oxidation-reduction potential. The upshot of the body of work e in the decade 1920–1930 (summarised by Chambers[3] and by B. Cohen) was stablish that the E_h of egg-cells under aerobic conditions is of the order of ·07 volt and under anaerobic conditions of the order of −0·17 volt. The ectations once entertained as to the value of a knowledge of the overall uction potential of the embryonic cell have not yet quite materialised, and knowledge of the properties of heterogeneous systems must, as Korr[2,3] points , advance a good deal further before this line of approach will again become ortant.

nalogous work is that on echinoderm eggs dealing with surface tension S. Cole[2]; Cole & Michaelis; E. N. Harvey[1,2,5]), cataphoretic charge (Uspenia; Dan[1,2,3]; Dan, Yamagita & Sugiyama; Mazia[1]), and electrical conductivity impedance (K. S. Cole[1,3,4]; Cole & Cole; Cole & Curtis; Cole & Spencer). It not be summarised here. In the case of the hen's egg physical methods have ved of value in studying the changes in water distribution. Orrù[1,3] and Romanov omanov[2], who confirm older work (*CE*, p. 878), show that the egg-white is tinuously dehydrated, partly by loss through evaporation, and partly because of uptake of water by the yolk which later loses it to the embryo. The changes lectrical conductivity and refractive index of the egg-white are therefore very siderable (Romanov & Sullivan; Romanov & Grover; Romanov, Smith & livan; Perov[1]; Perov & Dolinov). Electrical conductivity of egg-white drops n a high level to 33 % of its initial value and then slowly rises; that of the yolk ts at a value lower than that of the white, drops to the same relative extent then rises to nearly its original value; that of the allantoic liquid rises to a teau, and that of the amniotic liquid has a sharp rise and then a fall to below values. Refractive index follows a course reciprocal to that of conductivity, rising greatly, then finally falling off.

·42. Carbohydrate metabolism

the course of the argument on the biochemistry of organisers in vertebrates 189 ff.) we have already had occasion to consider a number of features of the abolism of carbohydrates in embryonic life. Without repeating these here, efore, we must give a picture of this department of metabolism as complete ossible. It is convenient to begin with the situation in birds and mammals.

·421. Carbohydrate metabolism in the development of higher vertebrates

s may be seen in the former summary (*CE*, Section 8·1) the general course arbohydrate changes in the hen's egg during development was rather confused. re emerged the facts, however, that free glucose decreased to a minimum ut the middle of the incubation period and thereafter rose to some extent, le glycogen, present only in traces at the beginning of incubation, increased steadily as time went on, first in the yolk-sac and later in the embryonic h itself. More difficult to trace were the movements of the protein-combined s (ovomucoid and the albumens and globulins). These relationships have b clarified by subsequent work.

Donhoffer introduced a new method of fractionation of the carbohyd groupings, based on alcohol extraction of the whole egg, which he found contain about 290 mgm. total glucose/50 gm. wet wt. (approximately one without shell). The alcohol-soluble fraction, interpreted as all the free gluc starting at 110 mgm. (in the same units), falls to 10 mgm. at about the 9th d and rises to 20 at the end of incubation. The water-soluble fraction of the alco precipitate, interpreted as mainly ovomucoid glucose, begins at 90 mgm., si to 65 at the 9th day and thereafter returns to 90 mgm. by the end of incubati The water-insoluble fraction of the alcohol precipitate declines more or l steadily throughout incubation from 85 to 40 mgm. On the other hand, glycog steadily rises from 10 to 60 mgm. Hence the water-insoluble fraction of alcohol precipitate is regarded as the principal source of the glycogen form it is supposed to be sugar in protein combinations other than ovomucoid (p. 9). In view of what will appear later (p. 616), it is not without interest t the free sugar appeared to contain a certain proportion of fructose as well glucose[1], and this was confirmed by K. Yamada[1]. The fall of free glucose has a been confirmed by K. Takahashi[1] and Tateishi[1].

One of the most striking events accompanying this great fall is, as has long be known (*CE*, p. 1051), the peak of lactic acid, both in yolk and white, wh accumulates till the 5th day reaching a maximum of some 80 mgm. %. K. Ta hashi[1] studied the effects on this of injecting carbohydrates and hormones, b even when glucose is injected the peak is quite unaffected (cf. the work on *in vi* autorespiration, p. 592). Glutathione appears to reduce it slightly, and t injection of 0·2 c.c. 1 % iodoacetate or bromacetate will halve it.

The rise in blood-sugar during the chick's development, mentioned in C p. 1039, has been further established by Milletti and Zorn & Dalton.

It is now agreed that glycogen appears among organs in the chick embr first in the heart (Jordan) and only at the 7th–8th day in the liver, up to wh time it is mainly in the yolk-sac (Dalton[4]; Muglia & Masuelli; Guelin-Schedrina Its appearance cannot be accelerated by injections of glucose into the e (Canevazzi). The earlier conclusion, represented in the review of M. Aron, t it appears first at the 11th–12th day in connection with the beginning of insu secretion* seems to be wrong, for Guelin-Schedrina[2] injected insulin into t embryonic blood circulation on the 5th day and yet found no liver glycog present till its usual time, the 8th. She also transplanted 3-day embryos to t chorio-allantoic membranes of older embryos whose blood certainly contain insulin, but although it was proved that functional vascularisation had occurr liver glycogen never appeared in the grafts before their presumptive time (anot case of physiological *herkunftsgemäss* behaviour). By the last week of incubati ogen is everywhere, though at hatching, liver glycogen rapidly disappears ell-known fact confirmed by Muglia & Masuelli and Trotti[2]).

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* In some mammals the insulin content of the developing pancreas seems to be exception high; in cattle up to 15 times that of the maternal gland, if Fisher & Scott are right.

he effect of endocrine factors on the glycogen of the chick embryo's liver been studied in an interesting paper by Gill, who used the Linderstrom-g-Heatley methods. Adrenalin, injected into the embryonic circulation any after the 11th day, caused a well-marked fall of liver glycogen, but when the cells were growing in explants the adrenalin effect could not be obtained, ough anaerobiosis caused complete breakdown of the glycogen contained in explants. The reason for this difference is not yet clear. Adrenalin* does reduce the muscle glycogen of the embryo until the 18th day. Amylase is to increase in activity during the development of the chick embryo as a whole lobkova) and its liver (Galvialo & Goryukhina).

he glycogen of the mammalian foetus and placenta is also less available than of the maternal organism. Insulin has relatively little effect on the foetal ogen stores in the dog (Schlossmann[4]) and the sheep and goat (Passmore & lossmann). In the rat, adrenalectomy, as Corey[1] found, depletes the maternal glycogen much more than that of the foetal liver, and neither that nor any r deviation from normal conditions has any effect on the placental glycogen. maternal liver glycogen, foetal liver glycogen rises to a maximum after ing, then falls off again (Stuart & Higgins). But on fasting it never falls so (remaining e.g. at 5 % instead of 0·3 %), and on breaking the fast, it rises h further than the maternal liver glycogen. It is striking that Passmore & lossmann observed in ungulates, where the foetal blood-sugar is normally er than the maternal, that in hyperglycaemia glucose goes through the barrier afterwards the levels settle down to their normal inequality. The glycogen he human placenta cannot, according to Rabe, be increased by hyper-aemia. Nor can it be decreased even if there is dire need for carbohydrate he maternal side, as Rabinovitch showed in his study of placental glycogen Caesarean section on diabetic pregnant women. Moreover, glycogen pears much more slowly in autolysis of the foetal liver than in that of the rnal (Aloisi[2], working on the guinea-pig), while the glycogen of the human nta gives only glucose and no lactic acid on autolysis, thus resembling the (Davy & Huggett). The conception of the placenta, and also the avian and lian yolk-sac, as the "foie transitoire" of Claude Bernard (*CE*, p. 1018), ins indeed classical (cf. the recent series of glycogen estimations in man by , in the rat by Corey[3], and in the rabbit by Loveland, Maurer & Snyder). e is, however, the suggestion of Szendi[3,4] that in some mammals the lung, the placenta, holds more glycogen than the liver at early stages, and acts as re until the liver can take on its function. Lastly, physico-chemical differences een glycogen preparations from embryo and adult have been described by Lund, mainly as regards optical rotation.

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ta on the onset of function in the adrenal gland will be found in the papers of Cattaneo[1] and ian & Derbyshire for the guinea-pig; S. Davies for the cat; and Pankratz for the rat. Lewis lling noted that the adrenal gland cells of the chick embryo continue to form adrenalin in culture.

Precursors of glycogen have been studied by injecting various carbohydrat into the hen's egg by Kataoka. Glucose, fructose, mannose, galactose, malt and lactose were effective; pentoses and glucosamine were not. Histochemic work on glycogen distributions in tissues, mostly of late embryonic life, alwa continues; some references are given for those interested (chick, Morell rodents and carnivores, Aloisi[3]; Komatsu; Manai; human, Aloisi[1]; Mitan N. Nakamura). Glycogen may have a connection with the calcification of bo (see p. 556).

3·422. The phosphorylation problem

The question next arises whether glycogen is really an immediate source energy for the embryonic tissues, or a remoter store. It arose originally in slightly different way. As we have seen (p. 603), there is much eviden for a succession of energy sources during embryonic development, carbohydra preceding protein and protein preceding fat. In casting about for the meanin of this, it was natural to wonder whether perhaps the breakdown of glucose mig not be a simpler mechanism than any of the others and hence would have onto genetic priority. If deamination and desaturation were out of court, glucos might be broken down in some simple way. Hence much effort has been devote to determining whether the embryo, like the classical examples of muscle an yeast, phosphorylates its carbohydrate, or whether some simpler mechanism takes the place in it of the complicated cycles now known to exist in phos phorylating tissues (cf. Parnas; Parnas & Ostern; Meyerhof[2]; D. M. Needham[2]

The first observation (J. Needham & Nowiński) was that the substrate pr ference of the 4-day chick embryo under anaerobic conditions is very narrow for besides glucose, mannose is the only carbohydrate which can be glycolysed any substantial degree.* All phosphorylated hexoses and glycogen are com pletely unattacked, though an occasional experiment may show a slight breakdow of glycogen or hexosediphosphate. In such cases there are indications of sum mation with the glucolysis proceeding at the same time, suggesting a separa pathway. Mannose and glucose compete. Up to the present time no one h succeeded in preparing actively glycolysing extracts of the chick embryo, parall with muscle extracts, but the glucolytic activity of brei is very considerable. Th substrate preference is common to all parts of the embryo, and is shown by br as clearly as by the intact embryos.

The chick embryo in the first week of its development thus joins that class tissues, of which tumour and mammalian brain are the most classical example which strongly attack glucose and leave glycogen and the phosphorylated hexos untouched. This division of tissues was first made by Bumm & Fehrenbach, i 1930; we shall shortly meet with it again. In this connection Schönfelder's wor is important. Reviewing the relevant physiological literature, he showed tha foetal skeletal muscle, the muscles of young animals just after birth, and d

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* This may be significant in view of the mannose-containing trisaccharides which, as we hav seen (p. 9), are prosthetic groups of egg-proteins.

vated adult muscle, have several common properties. Thus contractures are
en with nicotine, coniine, etc., a special type of tension curve is seen, and
overy is very slow.* No histological peculiarities exist, but the muscles are
arly characterised by the difference in their utilisation of substrates; thus
scle of the foetal type glycolyses glucose, not glycogen, as opposed to ordinary
letal muscle. Schönfelder found that the change-over occurred during the
t fortnight after birth in the rabbit. With Schönfelder's results may be com-
ed those of other workers (Koschtojanz; Koschtojanz & Rjabinovskaia), who
w that the phosphagen-content of foetal mammalian muscle is exceedingly
or even (Rjabinovskaia[1]) non-existent. Needham & Nowiński found that
ck leg muscle from the 15th day of incubation still showed glucolytic, not
cogenolytic, properties. They did not believe that the difference could
due to difficulties of permeability, as the brei was made by grinding
l cytolysis in distilled water. Moreover, as will shortly be mentioned,
osephosphate is formed from hexosediphosphate even when intact embryos
used.

ollowing the important discovery that *dl*-glyceraldehyde has the property of
npletely inhibiting anaerobic glucose breakdown even in very small concen-
tions (Mendel; Mendel, Bauch & Strelitz), while having no effect upon
osphorylated glycolysis, Needham & Nowiński found that the glucolysis of
chick embryo is also powerfully inhibited by *dl*-glyceraldehyde. It was
r shown (Needham & Lehmann) that the inhibitory effect is due to the
ompound only, which corresponds in its configuration to the *l*-lactic acid
iving from *d*-glucose in the body. Subsequently this was confirmed on
er tissues by Mendel, Strelitz & Mundell. Contrary to various statements
the literature, the discriminatory value of glyceraldehyde as between gluco-
s and phosphorylating glycolysis holds good if attention is paid to
ether the triose is present in the monomeric or dimeric form (Lehmann &
dham[2]).

he subject was carried further in a series of papers by Needham & Lehmann[1].
spite of the use of the most delicate methods available (the *B. influenzae*
ose dehydrogenase test and the mammalian malic dehydrogenase test),
nzyme I (the cozymase of Harden and v. Euler, see p. 209) could not be
onstrated in the chick embryo. On the other hand, coenzyme II (the
osemonophosphate codehydrogenase of Warburg) was found to be present
ll probability throughout development and even in traces in the yolk.†
sphorus-transporting coenzyme, presumably adenylpyrophosphate, could be
nonstrated in the embryo, but in very small amounts. A systematic investiga-
was then made of the enzymes involved in the phosphorylation cycles (see
. 322), and the effect of additions of all kinds of phosphorus transporters, such
adenylic acid, adenylpyrophosphate, and cozymase to the glucolysing but not
cogenolysing brei was tested. It was found that aldolase is present, but that

e also Rosanova; Arshavsky & Rosanova; Arshavsky & Malkiman.

treme lack of coenzymes in embryonic tissue has also been reported by Bernheim & v. Felso-
i working on the rat.

the triosephosphate formed by it from hexosediphosphate continually accum lates, even when intact embryos are used.* On the other hand, all the enzym concerned in the breakdown of phosphoglyceric acid are present in the embr All the facts established could be interpreted upon the hypothesis that in t chick embryo there are two separate routes of carbohydrate breakdown: (1) a n phosphorylating glucolysis mechanism, very active, and closely bound to the c

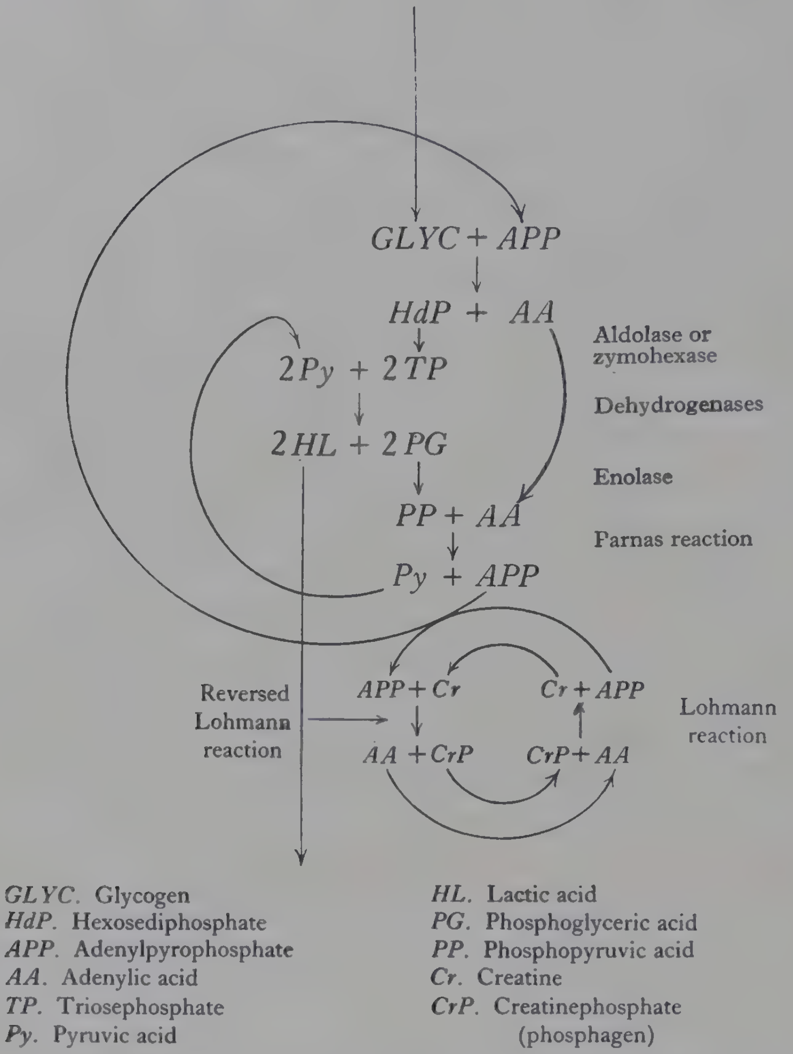
GLYC + APP
HdP + AA
2Py + 2TP
2HL + 2PG
PP + AA
Py + APP
Aldolase or zymohexase
Dehydrogenases
Enolase
Parnas reaction
Reversed Lohmann reaction
APP + Cr
AA + CrP
Cr + APP
CrP + AA
Lohmann reaction
GLYC. Glycogen
HdP. Hexosediphosphate
APP. Adenylpyrophosphate
AA. Adenylic acid
TP. Triosephosphate
Py. Pyruvic acid
HL. Lactic acid
PG. Phosphoglyceric acid
PP. Phosphopyruvic acid
Cr. Creatine
CrP. Creatinephosphate (phosphagen)

Fig. 322. Simplified diagram of the cycles of phosphorylating glycolysis. The important role inorganic phosphate is not shown (see D. M. Needham[2] and Pillai).

structure (desmo-enzyme), and (2) a phosphorylating system closely similar that in muscle, dealing with glycogen and hexosediphosphate, but of very lo activity because deficient in four distinct places, (i) the enzyme esterifyi glycogen, (ii) the enzymes forming phosphoglycerate and lactate from trios phosphate and pyruvate, (iii) lack of adenylpyrophosphate, (iv) lack of cozymas

* This step cannot be involved in normal glucolysis because normal glucolysis is inhibited *dl*-glyceraldehyde, and this inhibition can be reversed by the simultaneous addition of hexos diphosphate. The dihydroxyacetonephosphate formed from the latter removes the glyceraldehy giving hexosemonophosphate. It is therefore difficult to see how normal glucolysis, so ful inhibitable by glyceraldehyde, could involve hexosediphosphate as an intermediate.

eems, then, that in early embryonic life the phosphorylation machinery has yet been fully laid down.*

t is of interest that a not dissimilar state of affairs has been described for some ours. A number of investigators (e.g. Boyland & Mawson; Boyland & land[2]; Scharles, Baker & Salter) have found in tumour tissue the complete sphorylation system, as in muscle, but most of them admit that the course arbohydrate breakdown in tumour has certain peculiarities. Thus Caló and zuki found great inefficiency in the oxido-reduction between pyruvic acid triosephosphate; precisely the step which is missing in the embryo. chings, Oster & Salter have brought forward further evidence in support this and have shown that in presence of fluoride, muscle and tumour e quite different intermediate substances. Brain tissue is another case in t. Earlier workers (Ashford & Holmes; E. G. Holmes; Ashford) adduced y experiments in favour of two paths of breakdown there, glucolysis as well lycolysis of hexosephosphates and glycogen, and later successful extractions he phosphorylation cycle enzyme system were made (v. Euler, Gunther & tin; Mazza & Malaguzzi-Valeri). But Mazza & Lenti[1] went on to study the perties of brain brei from which all or nearly all of the phosphorylation vity had been extracted. The residue showed powerful glucolysis (as active n intact slices), inhibitable with glyceraldehyde, fluoride and iodoacetate, and ffected by addition of coenzymes or phloridzin. They were able to exclude hyl glyoxal and pyruvic acid as intermediates. In a later paper[2] they established fact that no esterification of inorganic phosphorus occurs when brain residue ns a vigorous glucolysis. Nor is any triosephosphate formed. Evidence for -phosphorylating glucolysis has also accrued for kidney and liver (Wajzer & pmann; Lenti[1]), for retina (Califano; Possenti; Lenti[3]) and for placenta tscher, Veith & Sarreither). Both brain and placenta contain strongly acting line phosphatases which would dephosphorylate phosphorylated hexoses schhacker; Busse).

rther experiments pointing in the direction of a non-phosphorylating lysis in the chick embryo were made by Needham & Lehmann[1], who found erential inhibition by fluoride. At *M* 200 the conversion of phosphoglyceric into phosphopyruvic acid by enolase, and hence the phosphorylation cycle, mpletely suppressed, while glucolysis in the same material is only 45 % ressed. Moreover, if by a carefully chosen fluoride concentration, the phorylation route is blocked but not the non-phosphorylation route, then nhibition of glucolysis should be greater in the presence of hexokinase than s absence, since the hexokinase should shunt the glucose into the phos- ylation route, where its breakdown beyond phosphoglyceric acid should be ossible. Conversely, glyceraldehyde will block the non-phosphorylation

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eyerhof & Perdigon later succeeded in obtaining breakdown of hexosediphosphate by chick yo by adding enormous quantities of coenzyme to replace the rapid destruction. Needham, ann & Nowiński then pointed out that embryo brei retains powerful glucolytic activity for g as 80 hr. at 0° although only a very short time would suffice for the complete destruction of enzyme present by the nucleotidases. Such preparations are absolutely incapable of carry- t the dismutation between pyruvic acid and triosephosphate.

route but not the phosphorylation route; hence in the presence of hexokinase inhibition of glucolysis should be less than in its absence, since all the gluc should be shunted through the phosphorylation route. Yet in all cases fluor and glyceraldehyde inhibitions were the same whether hexokinase was pres or not. This further strengthens the evidence that glucose breakdown in living embryo proceeds without phosphorylation.

In connection with the earlier results of Geiger with glutathione, it is interesti that Needham & Lehmann[1] obtained some evidence that glutathione can reve the inactivation of glucolysis brought about when embryo brei is dialysed. Th glutathione is the coenzyme of glyoxalase has been known since the work Giršavičius and Lohmann, but in the chick embryo methyl glyoxal cannot an intermediate stage in glucolysis, as Needham & Lehmann were able to sh that summation occurs when the enzymes are flooded simultaneously wi glucose and methylglyoxal.[*] It seems possible to exclude many other substanc also as intermediates, such as gluconic acid, glycerol, glyceric acid, pyruvic ac and dihydroxyacetone. Additional evidence favouring the non-phosphorylati scheme was obtained by Needham, Nowiński, Dixon & Cook in a study of th partition of the acid-soluble phosphorus at different stages of normal ar fluoride-inhibited glucolysis.[†]

Summing up, it seems clear that in the chick embryo during the first week its development, we have the most extreme case known of non-phosphorylatin glucolysis. This process is most active; phosphorylating glycolysis is weake In brain, tumour, etc. the two paths are more equally present. Two objectio against the non-phosphorylation hypothesis have been raised; it cannot accepted until (1) the enzymes responsible have been obtained in extract, (2) th intermediate stages have been discovered. The second objection appears to ha more force than the first, for many enzymes (such as cytochrome oxidase, f instance) are closely attached to the cell structure and have long defied attemp to get them off it.[‡] There is little that can yet be said about the intermediates non-phosphorylating glucolysis, but in spite of some serious arguments to th contrary perhaps the most probable suggestion is that the glucose molecule split into two molecules of glyceraldehyde.[§]

[*] The old observation of Neuberg, Kobel & Laser that methylglyoxal is formed from hexos diphosphate by chick embryo acetone powders was certainly due to the non-enzymic conversio of triosephosphate.

[†] This may be the place to mention a remarkable peculiarity in the distribution of acid-solub phosphorus in the chick embryo, namely the high proportion (56·5 %) not precipitable wi barium. This fraction, which is very resistant to acid hydrolysis, is not fermentable by yeast, an probably plays no part in the mechanism of carbohydrate breakdown. During later developme it declines relatively in amount. It is interesting that a fraction with similar properties and al present in large amount occurs in tumours. The nature of the material is still obscure; it may the aminoethylalcohol-phosphate of Outhouse; the diphosphoglyceric acid of Greenwald, or th choline phosphate of Beznák & Chain and Plimmer & Burch.

[‡] Lenti & Fuortes have made the interesting discovery that the enzymes of the phosphorylatio cycles are not destroyed by subjection of tissues to liquid air, but that those of glucolysis ar Presumably this is because the structure is destroyed and with it any enzymes attached to it.

[§] There are reports of the isolation of a non-phosphorylated compound intermediate in glucolys (Mazza & Lenti[2] on brain; Lenti[3] on retina); further progress will be awaited with interest.

)uring the investigations just described, the behaviour of pyruvic acid during ›ryonic glucolysis was examined by Needham, Nowiński, Dixon & Cook.* eems to be more concerned with autoglycolysis than glucolysis. They also sured the rate of oxidative disappearance of lactic acid and found that it is ıfficient to account for the effect of oxygen in reducing glycolysis. Meyerhof's nthesis theory cannot therefore explain the Pasteur effect in the chick embryo, ch must more probably be ascribed either to an action of oxygen upon ssibility (K. C. Dixon) or upon the enzymes themselves (Lipmann[3]).

e may now turn to questions relating to phosphagen (see the lower part of diagram in Fig. 322). Phosphagen (whether phosphocreatine or phospho- nine) is closely connected with the phosphorylation scheme of carbohydrate ıkdown, but it is now known not to be exclusively concerned with muscular ion, as was first thought. One of the most important ways in which this gy reserve is synthesised in the cell is by the Parnas reaction (Ostern, anowski & Reis; D. M. Needham & van Heyningen; Meyerhof & Lehmann), hich phosphopyruvic acid gives up its phosphorus to adenylpyrophosphate and atter hands it on to the guanidine base. Under suitable conditions tissues which tain hardly enough phosphagen to measure satisfactorily may thus be made to ıonstrate that they can form it (e.g. Baldwin & D. M. Needham[2] on echinoid muscle; Torres on many mammalian tissues). Baldwin & D. M. Needham[1] first wed that phosphagen is present in the chick embryo as early as the 70th hour evelopment. Very shortly after its first appearance in measurable quantities, tains a maximum (as related to total inorganic and labile phosphorus); this ıcides exactly with the first beginnings of movement in the embryo, estab- ed by the work of Kuo[1] — head vibrations, body vibrations, head lifting head bending. The heart beat alone has begun much earlier, about the hour. Later the phosphagen "grows" first tachyauxetically ($k = 1{\cdot}57$) then lyauxetically ($k = 0{\cdot}90$). When Lehmann & Needham[2] found the Parnas ion to be fully present in the chick embryo (see above) it was of interest to it back as far as possible, and it was found to be very active already at the hour when the heart is beating, though only feeble at the 23rd while the al folds are still open. Further research will be required to ascertain how significance attaches to the appearance of the Parnas reaction at the same as the heart beat.

om the point of view of recapitulation it is interesting that not even at the est stage investigated could any phosphoarginine be formed by the Parnas ion. This does not support the claim of Bagdassarianz that phospho- ıine is present in chick and amphibian embryos. Avian yolk, according to akahashi[2], contains a little phosphocreatine, the meaning of which is unknown. embryonic brain considerable changes in the proportion of adenylpyro- sphate with age were found by Epelbaum & Khaikina.

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this connection it should be remembered that Blazsó and Banga could demonstrate no vic dehydrogenase in embryonic muscle.

3·423. Ascorbic acid

Another subject which requires treatment under the heading of carbohydra is the metabolism of ascorbic acid (vitamin C; formula on p. 266). A vitamin some mammals, it might better be called a hormone for birds, since they certainly synthesise it, probably from glucose or other hexoses. It was first fou that the hen's egg at the beginning of development is devoid of ascorbic a (Hauge & Carrick; Machida & Sasaki), and the synthesis during development then followed by S. N. Ray and later by Martino & Bonsignore and Suomalaine This proceeds steadily; thus at the 4th day there is 0·012 mgm./embryo, and the 12th, 1·26 (see p. 335); and it is doubtful whether ascorbic acid shows bradyauxesis of the other highly reducing substance, glutathione, which is usua estimated at the same time (Bierich & Rosenbohm). Nor is it concerned w hereditary size differences (see p. 426).

It seems, moreover, that the chick is not the only organism capable of sy thesising ascorbic acid. The amphibian embryo does so too (Suomalainer Moreover, it is said that mammals also possess the power in foetal life and afte wards lose it. This was first inferred by Mouriquand & Schoen from the resistan of pregnant guinea-pigs to scorbutic diets, but was later proved more directly a number of workers (Rohmer, Sanders & Bezssonov; Rohmer, Bezssonov Stoerr; Giroud, Ratsimamanga, Rabinowicz, Santos-Ruiz & Cesa). From oth work (Neuweiler[1,2]; Goldstein & Volkenson; Ammon; Caffier & Ammon) it clear that the placenta contains appreciable amounts of ascorbic acid, but in relati amount it lags far behind the corpus luteum, which, as Biskind & Glick ha found, is second only to the pars intermedia of the pituitary and perhaps t adrenal cortex itself, where Szent-Györgyi first recognised the substan (Bourne[1]; Glick & Biskind). As the ascorbic acid content in the corpus luteu parallels the progesterone content, it is thought that the vitamin may be necessa for the production of the hormone. If so, it would have an important functi in maintaining successful pregnancies. Bourne[2] induced corpora lutea artificial by treatment with anterior pituitary hormone; the guinea-pigs were then mo resistant to scorbutic diets than their controls.

3·424. The fructose problem

Before leaving the embryos of birds and mammals, something must be sa about the curious association of fructose with embryonic life. Since the time Claude Bernard (1855) it has been known that the sugar of the human amnio liquid is laevo-rotatory, and as previously stated (*CE*, p. 1054) fructose is cor monly found in mammalian foetal blood. It also occurs in traces in norm blood (Okamura[1]), where it is said to be raised somewhat in amount duri mammalian pregnancy (Okamura[3]) and during the breeding season of the salm (Okamura[2]). Itizyo's work indicates that about one-tenth of the human amnio sugar is fructose, and the ubiquity of fructose was further established wh K. Yamada[1] showed that it is definitely present in the amniotic liquid of t chick embryo. As mentioned above, the chick embryo cannot glycolyse fructo

eedham & Nowiński). In order to probe further into its function Dickens &
eville[1,2] undertook an elaborate study of the fructolytic power of the embryonic
ffolding structures in mammals and birds. It was found that a fructolysis as
ense as the glucolysis exists in the amniotic membranes of rat and guinea-pig
d the rabbit allantois, and to a lesser extent in placenta of the ferret and the
centa and yolk-sac of the cat. The placentas of rabbit, guinea-pig, rat and
use, and embryonic tissues in general, do not fructolyse.* Without going into
further complications, the general picture seems to be that fructolysis is
racteristic of foetal scaffolding structures. It is regrettable that Dickens &
eville did not test the chick embryo's yolk-sac. And in view of trophoblastic
ctions it may be significant that tumour tissue also shows fructolysis.

3·425. Carbohydrate metabolism in the development of lower vertebrates and invertebrates

We come now to carbohydrate metabolism in the eggs of lower vertebrates and
ertebrates. Taking first the amphibia, reference must again be made to the
tails already given in connection with organiser phenomena, the glycogen
akdown, its distribution in the gastrula, and the rates of catabolism under
ferent conditions in the different regions. That glycogen undergoes a general
ninution all over the embryo throughout development is clearly established
rachet & Needham[2] on *Rana*; Kataoka & Tsunoo on *Cryptobranchus*; Taka-
atsu[2] on *Hynobius*). In the first of these cases it falls between fertilisation and
tching from 3·25 mgm./40 eggs to 2·15 mgm. and at the same time the propor-
n of desmo-glycogen becomes smaller (8·9 to 2·6 %). Total carbohydrate, as
vage[2] has shown, also falls. Expressed as loss/embryo in mgm. this varies from
0·065 in the case of *Rana*, *Bufo* and *Discoglossus* to −0·15 in the case of
mbina — somewhat greater than the glycogen losses just referred to.

Nowiński[3] extended the investigation of the course of carbohydrate breakdown
mphibian development. Taking larvae between loss of yolk and beginning of
ding, he established that there is the same substrate preference as that shown
the chick embryo, glycogen and hexosediphosphate being unattacked and
cose strongly broken down. This glucolysis was also inhibitable by *dl*-
ceraldehyde. Tadpole autoglycolysis, like that of the chick, decreases with age.
may be added that an amylase acting on glycogen and a glycerophosphatase
e been found in amphibian eggs by I. Takahashi[2] and by Takamatsu[1].

As regards phosphorus compounds, a phosphagen, later shown to be identical
h phosphocreatine, was found in frog eggs at fertilisation to the extent of
16 mgm./100 eggs by Zieliński[1]. About the 55th hour of development, during
closure of the neural folds, the phosphagen reaches a minimum of about
08 mgm./100 eggs, after which it rises to 0·05 mgm. at hatching. A similar
e occurs in fish embryos (Rosenfeld & Bagdassarianz), and the whole course
events in the frog embryo was strikingly confirmed by Verjbinskaia. Total
d-soluble phosphorus rises much further and more continuously, as also does

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There is, however, the observation of Watchorn & Holmes that the ammonia and urea pro-
ction of explanted embryo rat kidney is reduced in presence of fructose.

the inorganic phosphate, derived, as we shall see (p. 637), from breakdown of lip substances. The late rise of phosphagen accompanies the onset of muscul motion, but the meaning of the initial fall remains obscure. It might be tempti to relate it to the energy requirements of neural induction. Zieliński[1] also show that the amphibian embryo contains a good deal more creatine than that whi is present as phosphagen. His determinations of the partition of the acid-solub phosphorus were extended by J. Brachet[22] and Verjbinskaia to the period coveri gastrulation, but no marked variations were found to occur during that proces But the rise in total acid-soluble phosphorus, which goes on throughout develo ment, is almost entirely due to the difficultly-hydrolysable fraction which in th neurula accounts for 55 % of the total (cf. the chick embryo, p. 614). Adeny pyrophosphate, however, is at the end of the yolk-sac period about double i original amount (Verjbinskaia).

In Dorris' thorough work[1] on the development of the digestive system i tadpoles, it was found that the first enzyme to appear is amylase. This preced the histological differentiation of the gut, whereas the appearance of pepsin an trypsin follow it.

The only study of carbohydrate changes in a developing fish egg is that o Hayes & Hollett on the teleost, *Salmo salar*. Glycogen is absent at the beginnin but increases steadily at the later stages, though not in the liver till after hatchin (cf. the "foie transitoire", p. 609). The free glucose concentration also rises These authors propose a correlation between cleavage type and the nature of th store of carbohydrate initially present. Holoblastic eggs (e.g. those of annelids insects, echinoderms and amphibia), according to all the data we possess, hav glycogen; meroblastic eggs (e.g. those of fishes and sauropsida) have free glucose which is more readily diffusible. There seems to be nothing against this interest ing suggestion, which is indeed supported by the abundant polysaccharide o molluscan eggs (see p. 29), except the glycogen of the meroblastic cephalopo egg, referred to in the following paragraph.

Among invertebrates it is also usual for glycogen to fall markedly durin development, but in some cases polysaccharide seems to be a more importan egg material than in others. There is agreement that in silkworm developmen the glycogen almost disappears, falling, e.g., from 1·2 gm. % to 0·05 (Tirelli[3] Minoya). Histochemical observations on nematode (Giovannola) and sea-urchi (Kurkiewicz) embryos confirm this, and in the grasshopper egg the free glucos falls during pre-diapause (D. Hill) resembling in this way the hen's egg an agreeing with the R.Q. data. For the sea-urchin we have also chemical estimation of glycogen by Zieliński[2] and Örström & Lindberg. The eggs of squids and crab also have large supplies, according to Chaigne (up to 1·5 %), and we are reminde of the work of F.May and his collaborators, (p. 29), which revealed the quantitie of galactogen amassed in the eggs of land-snails. A glance at Table 10 will show moreover, that the eggs of certain invertebrates do, in fact, consume a relativel large proportion of carbohydrate during their development.

On the other hand, there are a few reports to the contrary. Thus Dyrdowsk finds that glycogen does not decrease during early developmental stages o ıatodes, and Nouvel states that it actually increases during the embryonic elopment of orthonectid parasites of ophiuroids, such as *Rhopalura*.

'hosphorus compounds related to carbohydrate metabolism have been studied he developing orthopteran egg by Thompson & Bodine[2] and Steinhart, in that he squid by Needham, Needham, Yudkin & Baldwin, and in sea-urchin eggs Zieliński[2]. The most interesting of these papers is the first. Pyrophosphate ısphorus was found to be high at the beginning and to drop sharply till the inning of diapause, after which it remained constant till the end. Since the rease in adenylpyrophosphate-content closely parallels the falling course of respiratory quotient (see Fig. 312), these observations support the notion of ırbohydrate period in early embryonic life, but in this case under conditions h that the adenylpyrophosphate cannot be resynthesised, or not as quickly as ıld be necessary to maintain its original level. In the locust egg, according to inhart, there is twice as much adenylpyrophosphate at hatching as at the inning of development. In the squid embryo, *Sepia officinalis*, Needham, edham, Yudkin & Baldwin found phosphoarginine from the earliest stage mined (36 days). It increased tachyauxetically ($k = 1{\cdot}54$ on the wet wt.), ng peaks at various stages when related to total water-soluble phosphorus or rganic plus labile phosphorus. This increase resembles that seen above in the k embryo (p. 615) and must be analogous to the rise at the later amphibian ges. Zieliński's figures[2] for the sea-urchin cleavage stages again show the ıtively large amount of barium-non-precipitable difficultly-hydrolysable terial characteristic of chick and frog embryo and tumours. No significant nges were observable in inorganic or adenylpyrophosphate fractions, but it nteresting that after iodoacetate treatment there was no accumulation of osediphosphate or decrease of adenylpyrophosphate, only a diminution of rganic phosphorus and an increase in the non-precipitable fraction. This trasts with similar experiments on the chick embryo with fluoride (Needham, viński, Dixon & Cook).

ı locust eggs Steinhart could find no phosphagen till an advanced stage of elopment. But the preceding paragraphs have contained a number of indica- s that in early stages (chick, frog, grasshopper, etc.) phosphocreatine and ıylpyrophosphate seem to be functional. How this affects the possible general ırrence of non-phosphorylating glucolysis at those stages remains to be seen.

·43. Protein metabolism

everal aspects of the protein metabolism of embryos have already been sidered in relation to the cleidoic and non-cleidoic systems constituted by erent types of eggs (Section 1·64, pp. 50 ff.). Something will be said later 671) on the structural aspects of proteins. Here, therefore, we shall be mainly cerned with amino-acid and enzyme changes during development.

·431. Protein metabolism in the development of the higher vertebrates

'he first question which arises is that of the relative rates of utilisation of the ous proteins of the yolk and white, described on pp. 8, 15. There is work on this by Schenck, whose data, however, are rather erratic, and there is the less ambitio but more convincing paper of T. Onoe[2]. From this it seems that the vitellin ar livetin of the yolk are absorbed at about the same rate, while the vitellomuco (although there is very little of it) is absorbed at a more rapid rate during t second half of incubation. In the white, ovomucoid is absorbed much faster tha the albumens (measured as total coagulable protein). Precisely the same rel tionships hold for the egg of the marine turtle *Thalassochelys* (Kusui[2]; M Takahashi).

In the former survey of these questions (*CE*, Section 9·2) the rise and fa of certain amino-acids in the whole closed system of the hen's egg was discusse The problem is a fair one, but unfortunately the changes are relatively very smal and it is unlikely that any of the technical methods which it has been possib so far to apply to them are capable of registering them accurately. In spite much contradiction, however, the general picture appeared to be a decrease i the monoamino-acids and an increase in the diamino ones, together with specif decreases in tryptophane, tyrosine and cystine, and increases in histidine ar lysine. But this is still subject to controversy, as the work of Calvery[3] shows; h believes that he has established a decrease in histidine. A great deal of labou was devoted by Schenck[1] to the analysis of protein samples from the whole embry the yolk and the white, at different stages of incubation, in order to prove th the amino-acid composition of the same protein varies during development. Th would have fitted in with other studies by the same author in non-embryologic fields (Schenck[4]; Schenck & Kunstmann; Schenck & Wollschitt), but his tech nique was strongly criticised by Abderhalden and he cannot be said to hav demonstrated either chemically or statistically that the variations he found we beyond the range of experimental error. The same criticism applies to his clai that the composition of feather keratin varies during embryonic development.

Work of a somewhat more careful kind was that carried out on the developin pig embryo (0·313 to 725 gm. wet wt.) by Wilkerson & Gortner, who used th van Slyke nitrogen distribution method on hydrolysed embryo acetone powder Amide, humin and cystine nitrogen showed no significant change. As in th whole hen's egg and in the eggs of salamander and teleostean fish (*CE*, pp. 11 and 1112), there was a slight decrease in the monoamino-nitrogen. Argini histidine and tyrosine decreased, lysine increased; though caution should b exercised in accepting these statements, since the powders used were not purifie proteins.

Wilkerson & Gortner were interested in the relative decrease in arginine whic they observed in the embryo pig because they regarded it as possibly of r capitulatory significance, since tumour protein (Edlbacher) and proteins invertebrates are often said to contain much arginine, though this is not in acco with the determinations of Rosedale & Morris. A similar decrease of arginine the developing chick embryo is reported by Rashba and Palladin & Rashba, b it would hardly be justifiable to regard it as yet established. That the enzym arginase decreases in activity during embryonic development was first notic by Edlbacher & Merz on the guinea-pig, and later fully established on the chi (Brachet & Needham[1]; Needham, Brachet & Brown), where it "grows" brad tically with a *k* of only 0·48. The latter authors also showed that this decrease rginase activity was not due to a diminution of activating substances, such as athione or ascorbic acid.

njections of arginine into the hen's egg by Kamachi[2] led to increases in histidine, the experiments were few and unconvincing. So also is the claim of Gold- ger that the 7-day chick embryo contains no free amino-acid nitrogen. This ect deserves much more attention than it has yet received, for the problem hether the proteins of yolk and white are converted into those of the embryo, or without complete scission to free amino-acids, is a very real one. Perhaps use of isotopes will help in its solution. Deuterium (Ussing[2]; Krogh & ing[2]) and isotopic nitrogen (Schoenheimer & Rittenberg[2]) are built into the ein of the embryonic body.

inally, proline and oxyproline manifest no change during the development of chick embryo (R. Ido). Alone among amino-acids, arginine increases the tinine of the egg (I. Takahashi[1]) when injected, and another Japanese worker, Ono, states that injected creatinine is destroyed.

oming now to the question of nitrogen excretion during the development of s, there is little to add to the previous discussion (*CE*, pp. 1076 ff.). The essive production of ammonia, urea and uric acid remains a remarkable , but it would be very desirable to know to what extent the extra-embryonic nbranes contribute to the nitrogen excreted, and in what form. Nothing on e lines has yet been attempted. How exactly the catabolism of protein inates in the chick embryo is unknown; the *in vivo* maximal ammonia luction occurs on the 4th day, that of urea on the 9th and that of uric acid he 11th (see *CE*, Figs. 323 and 324). When the embryo of the 4th–5th day is lied *in vitro*, as by Dickens & Greville[3] and by Needham[8], the ammonia luction is considerable in the absence of added substrate (glucose) and the is 0·75, but in its presence the R.Q. is unity and the ammonia production very l. Thus the embryo at this age certainly has the ability to catabolise protein, shown further by the fact that if fluoride is given as well as glucose, the onia production rises to a level just sufficient to account for the whole of espiration as protein catabolism, though the R.Q. is in this case unchanged dham[8]). But it had already proved impossible (Needham[2]) to postpone the ıal rise in nitrogen excretion by injecting glucose into the egg at the begin- of development. There is therefore some intrinsic mechanism governing transition from carbohydrate to protein catabolism, of whose nature we are ignorant.

n this connection we cannot accept the contention of Glover that no deamina- of amino-acids by 5-day chick embryo tissues takes place, all the more so his is not borne out by his figures, which do show a rise of ammonia above controls, ranging from 12 % in the case of glutamic acid to 77 % in the case roline, with an average of 33 % for all amino-acids. No doubt the deaminases weak. One curious thing about the "protein-sparing" action of glucose in kens & Greville's experiments[3] is that it is shown also by fructose, a carbo- rate which is not anaerobically converted to lactic acid by the embryo

(Needham & Nowiński). Rat embryo tissues, according to Dickens & Grevil behave in a similar way to the chick embryo, but the rat yolk-sac produc practically no ammonia whether glucose is present or not, though in absence glucose its respiratory rate does not fall. This is unexplained. Jensen rat sarcor behaves like the chick embryo.

If ammonia production may be assumed to be by direct deamination, the was room for much speculation regarding the origin of the chick embryo's ure till Needham, Brachet & Brown showed that it arises, not from general prote catabolism by way of the ornithine cycle, nor from uric acid by Stransky complex (uricase, allantoinase and allantoicase, see p. 52), but entirely from t arginine-arginase system. The embryo thus "recapitulates", not the ornithin ureotelism of amphibia and chelonia, nor the uricase complex of amphibia ar fishes, but rather the arginase system itself, widely present throughout inverte brates (Baldwin[1]). The production of urea from arginine by arginase is also a adult avian characteristic, and is the sole mode of origin of urea in adult bird It was furthermore shown by Needham, Brachet & Brown that the urea forme cannot participate in the synthesis of uric acid by the embryo, in agreement wit the views of Clementi[2] and his school. By what means the uric acid of the embry is synthesised remains mysterious, but a discussion of this process in adu sauropsida has already been given (p. 55) in connection with the cleido question, to which the reader is referred. During development the blood ur acid, measured by Zorn & Dalton, slowly but steadily rises.

Nitrogen excretion in the chick embryo has, of course, morphological aspect Boyden[2] was able to correlate the development of the kidney with the developmen of nitrogen catabolism. Uric acid excretion begins on the 5th day. By the 13t day the allantois contains between 8 and 13 mgm. uric acid dissolved in 6 c. water. The mesonephros is therefore clearly functional. Now between the 5t and 14th days the whole Wolffian body increases its volume 12 times. Thoug there is no increase in glomerular count, the tubule volume increases 16 time the corpuscle volume 3 times, the collecting tubule volume 10 times but th secretory tubule volume 33 times. The glomerular volume of the 14th da mesonephros is 50 times that of the metanephric glomerulus at the same ag Thus the growth of the chick embryo kidney during the main period of nitroge excretion (the protein-combustion phase) is located in the secretory portion the tubules. In 1931 it was suggested by Needham[4] that all the uric acid in th allantois is free. Thus the pH of the allantoic liquid about the 15th day is 6·3, contains 10 times too little ammonia to account for the acid as ammonium urat and the inorganic anions balance the inorganic cations (Kamei; Iseki[1]). Ther might thus be a cycle of base as well as water in the conveyance of uric acid int the allantois, similar to that shown to occur in the Malpighian tubes of insects b Wigglesworth[1]. Uric acid is deposited in the form of crystals during the latt half of incubation, rendering the allantoic liquid milky, and these are rendere harmless by the mucus which Rossi's allantoic vesicles secrete. The existence the cycle of base was later demonstrated by Białaszewicz & Głogowska, wh found that while the absolute amount of total nitrogen in the allantoic content ased steadily throughout incubation, the amount of sodium increased to ximum of 25 mgm. on the 12th day and then rapidly fell off almost to zero e time of hatching. It must be concluded that before the 12th day excretory esses predominate over absorptive ones as far as sodium is concerned, but after that time the opposite occurs. The potassium does not show this but increases steadily until at the end of incubation it reaches an amount out 7 mgm. The maximal volume of the allantois is reached at the same as the sodium maximum (10·3 c.c.); at the time of hatching it is only .c.

r mammalian embryos the placental route of excretion is, of course, open p. 1122), and the absence of urea from foetal bladders cannot therefore mean, houke supposes, that none is excreted by the foetus till birth.

he onset of function in the kidney has often been studied, and the most recent t of view will be found in the paper of Gersh. It is accompanied, according lexner & Gersh, by an unusually sudden rise in oxygen-consumption of the e, and an increase in histologically detectable cytochrome (Flexner[2]). The way excretion of sulphonephthalein dyes through the mesonephric cells overed by Chambers & Cameron was elegantly studied by Chambers & pton, who were able to show the accumulation of dye in explanted tubules h had spontaneously closed themselves. This transfer polarity could be royed by cold or respiratory inhibitors (Chambers, Beck & Belkin; Beck hambers), and it appeared later *in vitro* than *in vivo*. In fishes the ap- ch of blood-vessels to the tubules seems to be the most important limiting r (Armstrong). The pronephros of amphibia is capable of much more etory work than it ever has to perform under normal conditions, as is shown he parabiosis experiments of Hiller[3], in which only one pronephros could take of two animals.

s we have already seen (p. 72), the proteases of the yolk-sac are many times active than those of the embryo. The best study of the latter is that of kowski[2], who noted very little change in activity of the kathepsin of the yonic tissues during the development of the chick embryo, whether activated steine or not (P.Q. 0·27). At first the tail portion has more than the head, s time goes on, the enzyme becomes mainly localised in liver and kidney. kowski could not find any synthesis of protein by chick embryo kathepsin *ro.* On the other hand, a certain increase of kathepsin activity during the lopment of embryos has been reported by Kolobkova for the chick and by lstein & Millgram for mammals. By the use of micro-methods it has been lished that the ectodermal parts of the 3-day chick embryo head have three s as high a dipeptidase activity as the mesodermal parts (Levy & Palmer; ner & Levy). Enzymes of the intestinal tract and its derivatives would rally be expected to increase gradually during development, *pari passu* with istological changes, and this seems in fact to happen (Galvialo & Goryukhina hick embryo stomach proteases; Orechovitch[3] on chick embryo liver epsin and dipeptidase; Michejev on rennin in the stomach of cow foetuses; n, Jarmoschkevitch & Jakovtschuk and Cardin on the intestinal tract of human embryos). In the last-named case enzymes appeared in the follow succession: dipeptidase at the 2nd month; kathepsin at the 3rd; urease at 4th; and tryptic protease and carboxypolypeptidase at the 5th.

An interesting but isolated observation is that of asparaginase in chick embr liver at the 8th day by Impallomeni. Since suspicion has fallen upon glutami as concerned in uric acid synthesis, there is a way open here for some interesti experiments.

3·432. Protein metabolism in the development of lower vertebrat and invertebrates

Passing now to what is known of nitrogen metabolism in amphibia and fishe we find that the newer work is very fragmentary. That the eggs of all fishes ar amphibia excrete a certain amount of ammonia into the surrounding water h long been observed (*CE*, pp. 1132 ff.), but the only up-to-date work on th is that of S. Smith[1], who used the eggs of the trout, and Ivlev, who work on *Silurus glanis*. As already mentioned (p. 49) the trout embryo resembl other aquatic embryos in combusting a relatively large quantity of protei Its ammonia nitrogen excretion rises from $20–50\gamma/100$ eggs/hr. to $150\gamma/10$ eggs/hr., accounting for at least 80 % of the excreted nitrogen; the rest seem to include amino-acids, possibly arising from proteolysis of the egg-membran (cf. hatching enzymes, p. 627). Felix, Baumer & Schörner, also working on th trout, noted the presence of dipeptidase and arginase in the egg at the outse later falling in activity, and no tryptic protease, kathepsin nor polypeptidas The amino-acids arginine, tyrosine, histidine and cystine remained constant, bu there was a fall in tryptophane and lysine. Little advance has been made wit the metabolism of selachian embryos, but the presence of bases in dogfis embryos, such as betaine, choline and trimethylamine-oxide (already discusse in *CE*, p. 345), has been fully confirmed by Spahr and Kutscher, Muller Spahr.

In Section 2·351 we had occasion to say a good deal about carbohydrate —an even lipin — metabolism in the developing amphibian embryo with reference organiser phenomena. Protein changes have not been overlooked. According Graff & Barth, there are no changes in total cystine during cleavage, but betwee early gastrulation and hatching there is a large increase, perhaps at the expens of methionine. Arginine also increases, but very slightly. Analysis of the protei metabolism of the amphibian gastrula urgently needs continuing. In the e periments of Boell, Needham & Rogers, the anaerobic ammonia production the dorsal lip region was found to be three times as high as that of the ventr ectoderm:

	Ammonia production $\mu l. \times 10^{-3}/\gamma$ dry wt/5 hr.
Early gastrula ectoderm	0·97
Middle gastrula dorsal lip region	2·97
Neural tissue from open neural folds	2·70
Neural tissue from just closed neural folds	1·30
Later neural tube and somites	0·98

ammonia production, therefore, seems to be higher during gastrulation 1 just before or just afterwards. But this is not detectable when aerobic nonia production of the intact whole embryo is measured (J. Brachet[22]). ;. Pickford is studying the dipeptidase distribution in the regions of the gastrula; eems to be inversely proportional to the yolk-content, as would be expected n the results on echinoderm eggs (see pp. 419, 627). Ammonia is not the sole ogenous end-product of amphibian eggs (see *CE*, p. 1107). Takamatsu & nachi found 4 mgm. % of urea in *Cryptobranchus* embryos at the 4th week, only traces of uric acid; and Takamatsu[1] reports a similar result for *Hynobius*. F. Munro, in an interesting paper, has shown that the ammonia excretion, preinant in the tadpole, gives place during metamorphosis to a urea excretion opping from 90 to 19% of the total N), while at the same time there is a ntyfold increase in the liver arginase, presumably for the ornithine cycle p. 52). These changes, followed in *Rana*, *Bufo*, etc., do not occur in the nanently aquatic toad *Xenopus*.

s regards enzymic activities in the amphibian embryo, tryptic protease and nase, but not urease, were found in *Hynobius* eggs by Takamatsu[1], but none hese could be recognised in those of *Cryptobranchus* by I. Takahashi[2]. The t interesting study in this field was that of Truszkowski & Czuperski, who ld find no uricase in frog eggs, but observed its appearance during developit in two distinct increases, the first at the time of disappearance of the external , the second at the time of completion of limb-bud growth. The former den rise was associated with the onset of liver function, the latter with that he kidneys. Both organs contain uricase, of course, in the adult frog.

hen we come to the invertebrates, we find that interest centres mainly round forms, the silkworm moth and the sea-urchin, the one a typically terrestrial the other a typically aquatic egg.

1 the developing silkworm egg, by all expectations (see p. 56) uric acid ld accumulate. That it does so appears from the figures of a number of ers (Akao[3]; Tanda; Manunta[2]), although the production of uric acid seems much larger during larval (Kuwana[3]) and pupal (Brighenti) life. According anunta's figures[4], most of the uric acid present at the time of hatching is dy contained in the undeveloped egg, suggesting that the maternal organism sits it there. This curious result demands reinvestigation. So also does the stent statement, originating from the work of Ashbel[4] and Ongaro[3], that loping silkworm eggs produce as much as 0·5 gm. ammonia kilo wet wt./hr. is quite irreconcilable with uricotelic metabolism, especially as Manunta[4] d no trace of uricase in the eggs, and seems likely (if true) to be due to ictivities of the abundant bacterial flora with which, according to Masera, vorm eggs are provided. Until sterile silkworm eggs are available, this ition will not emerge from the state of uncertainty surrounding till recently iitrogen excretion of the blowfly larva (see p. 56). Lastly, an increase of in the developing silkworm egg has been reported by Bottero. This also d repay further investigation, as it resembles the appearance of urea during evelopment of the chick embryo, just discussed (p. 622). Another insect egg,

that of *Antheraea pernyi*, analysed by Leifert, showed uric acid as the overwhel ingly preponderant part of the sum of excretory nitrogen. And grasshopper eg (*Melanoplus*) lose no nitrogen during their development (Bodine & Tro bridge).

The developing echinoderm would be expected to give off a certain amou of ammonia, arising from protein breakdown. This it undoubtedly does. Ashb first and then Örström[3] found an excretion of ammonia amounting to 1 micromo c.c. eggs/hr. before fertilisation (*Paracentrotus*). After fertilisation, according Ashbel[4], the ammonia production continues unchanged, but according to Örströ it decreases to zero. Conversely, in anaerobic conditions the unfertilised eg produce 0·5 micromol, while the fertilised ones produce 1·3 micromols. Oth amines may also be excreted (Örström[3]).

But this is by no means the whole story. In 1933 Hayes[2], studying the Florid species *Echinometra lucunter*, reported a rise in the total nitrogen of the eg between fertilisation and the pluteus stage (see Fig. 323).* This was generally regarded at the time as incredible, but Örström[4] quite independently discovered in *Paracentrotus* eggs an ability to bind considerable amounts of ammonia from the environment. This binding capacity is in the neighbourhood of 3 micromols ammonia/c.c. eggs/hr. aerobically and 10 anaerobically for the unfertilised eggs, and no less than 50 micromols aerobically and 12 anaerobically for the fertilised eggs. The ammonia taken up goes into organic "unhydrolysable" combination, and the amount can be increased by the simultaneous addition of aspartic and glutamic acids, histidine and ornithine. Örström interprets these facts as meaning that an oxidative amination of keto-acids occurs after fertilisation. The full publication of his results will be await with interest; should they be confirmed, they would add a further remarkab chapter to the natural history of the cleidoic egg (pp. 34 ff.), for we shou have to admit that in some non-cleidoic eggs, dependence upon the enviro ment would go as far as a proportion of the potein-building element its Örström's work should be extended as soon as possible to eggs of invertebra species more primitive than the echinoderms.†

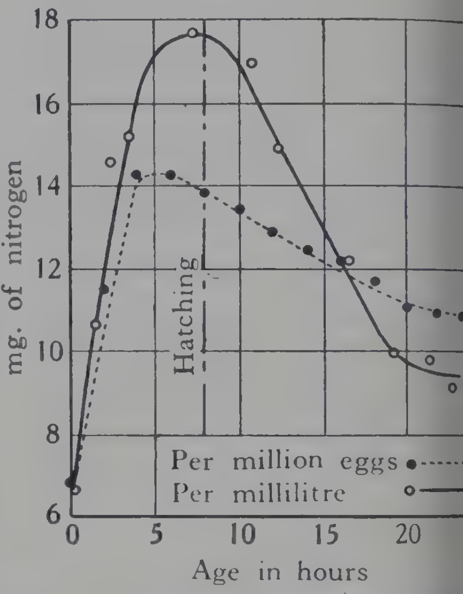

Fig. 323. Nitrogen content of the eg of the sea-urchin *Echinometra* (see tex

he nitrogen partition of the developing eggs of the gephyrean worm, *Urechis*, been followed by Horowitz[1]. One can hardly believe that no appreciable tein catabolism goes on in these embryos, as would appear from the stability he total nitrogen figures. Ought we perhaps to beware of compensation by a ogen intake here?

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* No such rise occurs during the development of *Urechis* from egg to trochophore (Horowit

† There is another case of this nitrogen-intake recorded in the literature, namely that of t eggs of the gastropod *Hemifusus tuba* reported by Kumon, and in the light of Örström's worl should certainly be reinvestigated. Organic substance was also found to *rise* slightly during velopment by Kamachi[3] on the cephalopod *Loligo bleekeri* and even by Takamatsu[1] on the amphib *Hynobius nebulosus*. There is now just a possibility that these workers were not mistaken; cf. t review of Krogh[2] on the dissolved organic substance of sea- and fresh-water.

nother direction in which results of much importance have been derived n experiments on marine invertebrate eggs is that of the location of intra-ular enzymes. Approaching the subject with the ultra-micro-chemical tech-ue developed by the Carlsberg school (see Linderstrøm-Lang & Holter[2]), derstrøm-Lang[1] in 1933 estimated the dipeptidase activity in two marine ertebrates, the gephyrean worm *Urechis* and the sand-dollar *Dendraster*. There eared to be twice as much alanyl-glycine dipeptidase in the latter as in the ner. When *Dendraster* eggs were cut into half, the activity of the two halves approximately equal to that of the whole egg, showing that the presence of nucleus was immaterial. On fertilisation in *Urechis* the activity fell, and later and fell again. The work was continued by Philipson, who found that the ribution of this enzyme was uniform among all the layers formed in *Psamm-nus* eggs by centrifugation. The conclusion, that this dipeptidase is associated the hyaline ground substance of the egg and not with any of the formed nents, was finally clinched by Holter[1] (using *Arbacia*, *Echinarachnius* and *etopterus*), and this fully agrees with the results of Holter & Kopać on the eba.* It seems that some egg dipeptidases need activation by magnesium; is so for leucyl-glycine dipeptidase in *Tubifex* eggs, but not for alanyl-glycine ptidase (Holter, Lehmann & Linderstrøm-Lang).

articularly interesting was the attempt to follow the dipeptidase through the ial determination periods. Doyle first found that there is no change in ptidase activity during fertilisation (*Psammechinus*), and Holter, Lanz & derstrøm-Lang then went on to show that all eight blastomeres at the 8-cell have the same amount of the enzyme. This is important, because the third age is equatorial (see p. 479) and separates the future endodermal rial and future micromeres from the future ectoderm. Finally Holter & ahl found that neither animal and vegetal halves of blastulae and gastrulae, ectoderm and endoderm of plutei, differ appreciably in their dipeptidase ity.

433. Hatching proteases

oteases of very specialised function are those which are secreted into the itelline cavity in nearly all phyla by embryos towards the time of hatching , p. 1595). These digest the protein of the egg-case or membrane. Proteolytic hing is of very wide occurrence; apart from examples previously given, it is rted, with much detail, for amphibia (Cooper; Nedler), selachian fishes ntrebert & T. Y. Wang; T. Y. Wang), teleostean fishes (Bourdin), ascidians rill[1]), orthoptera (Slifer[6]), cephalopods (K. C. Yung; Jecklin; Hibbard[2]),

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is is in contrast to the amylase of the amoeba which was shown by Holter & Doyle to be y associated with the mitochondria.

echinoderms (Ishida), and trematodes (Onorato & Stunkard). In many case the enzyme is secreted by special organs or glands which disappear after hatchin such as the organ of Hoyle in cephalopods or Stunkard's glands in trematode or the pleuropodia (organs whose function had been a mystery for a hundre years) in grasshoppers. The pleuropodia were proved by Slifer's ligature exper ments to be the origin of the hatching protease. Proteolytic hatching seems to a later development in evolution than the earliest method, osmotic hatchin (cf. *CE*, p. 1600), and it lasted on until the hard-shelled sauropsid egg necessitate more violent measures. This problem solved itself, however, since the cleido egg permitted the embryo to remain within it until it could hatch, as it does the gallinaceous fowls, in a state almost of adult perfection, and hence of con siderable muscular power. Moreover, as the review of Sergeiev reminds u there are special adaptations to the hard-shelled egg such as the transient ecto dermal proliferations known as "egg-teeth" or "egg-tubercles".

3·434. The growth-promoting factor

In a paragraph at the beginning of this section, reference was made to th problem of whether the proteins of yolk and white are broken down to simp amino-acids before being built into the embryonic body, or whether this trans formation takes place rather by way of intermediate substances of proteose typ This question would be important enough if its embryological interest alone wer considered, but it has also remoter repercussions.* The possibility arises tha such a proteose is identical with that which has been thought to be the cause the growth-promoting power of embryo extract. In order to explain this, a ver brief summary of the development of tissue-culture technique is necessary.†

R. G. Harrison's first tissue cultures[2] in 1907 were made with adult lymph, an it was Carrel[1] who introduced in 1913 the practice of using saline extracts or pres juices of chick embryos, which were found to be far more effective in promotin the growth of the explanted cells.‡ Since that time, the effect of age has bee studied in two ways, by varying the age of the tissue explanted, and conversel by varying the age of the donor of the extract. It may now be said that th effectiveness both of growth-promoting power, and of capacity to grow, de crease with advancing age. Taking the latter point first, Cohn, Murray & Rosentha showed that the growth capacity of cells from the chick embryo falls off ver regularly during development, while the latent period increases (see *Cl* Fig. 62§); this has frequently been confirmed (Hoffman, Goldschmidt & Do

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* It could almost certainly be solved by the introduction of amino-acids labelled with the hea nitrogen isotope into the hen's egg at the beginning of development, along the lines to be mentione later in connection with lipin metabolism (p. 638).

† For further details, consult the reviews of A. Fischer[4,5] and E. Mayer[2].

‡ Of course even in the presence of embryo extract cultures eventually cease to grow. Baker[2] h shown that this is probably due to an exhaustion of the nutrient materials in the serum, for grow will continue indefinitely if fresh serum is provided from time to time.

§ Significantly, it is said that cancer cells, when explanted, show practically no latent perio (Doljanski & Hoffman).

ki; Medawar*), but if the cells are grown for some time *in vitro* and sub-
ured, it can no longer be demonstrated (Parker[2]). But the former point is
more interest here. That the growth-promoting power of hen plasma
s off with age was proved by Carrel & Ebeling, and it does so more or less
passu with the cicatrisation power of wounds, the declining curve for which
worked out by du Noüy. Confirmatory evidence for this was provided by
ker[1] and others, and it became generally accepted that the period between
ıd 10 days of incubation was the optimum time for the preparation of the
ryo extract. However, this has since been modified by the elaborate work
aillard[1]; Andai; and Fowler, who agree that the maximal growth-promoting
er is reached not at that time but somewhat later, between the 11th and 17th
of incubation, though explanted tissues differ a certain amount as to the
mal age of the embryo providing the extract. Gaillard & Varossieau have
shown that the age of the donor embryo affects the direction in which *in vitro*
rentiation proceeds. It makes no difference from what region of the chick em-
the extract is prepared (Fowler; Hueper, Allen, Russell, Woodward & Platt).
Iuch work has been done on the distribution of the growth-promoting factor
its effects. It may be prepared from chick embryo blood (Zifferblatt &
laus) and in smaller amount from the maternal tissues of pregnant mammals
ntanni; Pybus & Fawns); it accelerates the growth of planarians (Pettibone
ulzen) but not that of mammals in the post-natal period (Kaufman[5]). Certain
lt tissues possess more of the factor than others, e.g. brain, spleen, thymus
thyroid (Trowell & Willmer; R. S. Hoffman). Pomerat & Willmer have
ght to explain this on the basis of a common possession of glucolysis inhibitable
glyceraldehyde (p. 611), but as the glyceraldehyde inhibition of tissue cultures
nspecific (shown by all aldehydes) it is not certain that this is a useful line of
ught. The growth-promoting factor in embryo extract can be completely
bited by lipin preparations, such as the petrol-ether extract of mammalian
used by E. Mayer[1], but this inhibition is not reversed by heparin. Indica-
of specificity are shown by findings such as that of Horning & Byrne, namely
issue juice of tumour-bearing animals is more favourable for *in vitro* growth of
our tissue than tissue juice from normal animals. The most interesting recent
ts here are those of Willmer and his collaborators, who have established that
ryo extract will start growth again after tissue in culture has stopped growing
lmer & Jacoby), and that the continued presence of embryo extract is not
ssary for the mitoses and migration which it produces (Jacoby, Trowell &
mer). Embryo extract speeds migration, increases the number of mitoses,
shortens the time taken by each mitosis. The presence of 15 % embryo
act in Tyrode solution for 1 hr. is sufficient to produce a large crop of mitoses
y hours later, and within limits the higher the concentration of the extract
shorter the time needed for its effect. If it is present for less than 10 hr. only
crop of mitoses occurs. This reminds us of the problem of the time taken by
nductor to produce its effect (pp. 156, 291).

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dawar[2] used an interesting method; he found that the younger the cells, the higher the dose
soderm-inhibitory factor of malt extract (see p. 359) was required to stop their growth.

What is the nature of the growth-promoting factor? It is partly, though no entirely, destroyed at 70° C. (Carrel[1]; I. Lasnitzki). It was early realised tha proteins alone would not do (Smyth; Swezy) and Baker & Carrel[1] went on to sho that mixtures of amino-acids were equally ineffective. Indeed, Mayer & Fische found later that after culturing on amino-acids alone, tissues even lose thei power to respond to embryo extract. Polysaccharides were ruled out by Hueper Allen, Russell, Woodward & Platt. But Carrel & Baker then discovered th important fact that peptic digests of proteins (e.g. egg-albumen, edestin, casein fibrin) showed considerable growth-promoting power, though by prolonge digestion this was destroyed. The activity of proteoses was later confirmed b Fischer & Demuth and Willmer & Kendal, though as they are ineffective in th absence of plasma or embryo extract,* it is clear that they require some furthe factor. This was conjectured to be the protease of the plasma, and following thi line of thought Borger & Zenker[1] obtained some experimental evidence that th growth-promoting power of embryo extract was proportional to its proteas activity.† On the other hand, when Borger & Peters examined the enzymes 10-day embryo extract, though dipeptidase and amino-polypeptidase were presen their activity was very weak, and no protease could be demonstrated. Later Hueper and his colleagues did find a weakly acting kathepsin. But it is no necessary to postulate protease activity; some carrier substance of nucleotide typ might meet the case as well or better.

The next important step was that taken by Baker & Carrel[2], who showed tha the growth-promoting power of proteoses (protein digests) plus thymo-nuclei acid was almost the equal of that of embryo extract. The level of embryo extrac was at last reached when Baker[1] made a mixture of casein digest, glycine, thymo nucleic acid, glutathione and small amounts of haemoglobin, but even here, th activity was not maintained for so long as that of embryo extract. Baker & Carrel further showed that α-proteoses and β-proteoses were equally effective, but afte this (1928) the work of the New York school diverged in another direction an this line of work lapsed. Although the success of the organ-perfusion metho (Carrel & Lindbergh) is striking and can lead to hyperplasia *in vitro* (Foot, Bake & Carrel[4]), the media used for it are very complex mixtures, including man vitamins and hormones (Baker & Carrel[4]; Baker & Ebeling), so that we obtain n further knowledge of the growth-promoting factor.

The subject was, however, revived in what may be important work b A. Fischer[7], of which only an abstract has yet appeared. From cow embryos h isolated by the Hammarsten method for nucleoproteins a substance which show great effectivity. The composition of this nuclein fraction seems to be unusua but it contains nucleic acids of both thymo- and phyto-nucleoprotein type, an it is with the latter of these that the growth-promoting power seems to b associated. If this is so, some light will be thrown on the occurrence of phyto nucleic acids in animal embryo tissue (Jorpes; Levene & Jorpes; Caspersson

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* Embryo extract need only be added to the proteoses at concentrations of 1 % or less to mainta full activity, suggesting the presence of carrier substances.

† Coagulation factors in embryo extract have been studied by J. T. King.

active substance is destroyed by short tryptic digestion and by boiling, but a
mostable substance (possibly the same as that described by Willmer & Kendal)
so involved. In the dried state the substance may maintain its activity for
y months (Borger & Zenker[2]; Peacock & Shukov; Hetherington & Craig).

e are thus led to a hypothesis which would link together many apparently
lated facts. Substances of nuclein type are now found to intervene, apart
their role in the cell nucleus, at many previously unsuspected points.
ain nucleotides in the cytoplasm are essential coenzymes in glycolysis,
sphorus transfer, hydrogen transfer, and amino-acid oxidation* (see p. 208).
y plant viruses, and the Rous sarcoma virus, and even a closely similar
tance which can be prepared from the chick embryo itself (see p. 269) contain
eotides. Proteoses plus nucleic acid are most effective, as we have seen, in
noting the growth of explanted cells. Is it not possible that a substance of
eotide structure might act either as a carrier for the requisite peptone
ndle" entering the embryo at the absorptive surface of the yolk-sac and so
ect it from the further action of proteases, or else possibly as a "bricklaying"
hanism at the site of protein synthesis, and that thus in combination with
peptones it might be in fact the growth-promoting proteose? Consideration
ch a question requires some knowledge of the behaviour of nucleins during
ryonic development, and this will be the subject of the succeeding section.
facts may, however, be noted at this point. First, the growth-promoting
r is to some extent dialysable (G. P. Wright; Tazima; Jacoby[2]) and although
day egg-yolk itself is inhibitory on account of the lipins in it, G. P. Wright[1]
able to prepare a very active dialysate from it. Secondly, the peptone bundle
red as the lowest building-stone of proteins must be quite small, since the
vth-promoting factor of embryo extract is quite non-species-specific (again
inductors), as Kiaer[1] and others have shown.

hat may be an important addition to the argument here is the work of
bourow and his collaborators on growth-promoting substances produced by
cells when damaged by ultra-violet irradiation. The wave-length most lethal
e cells is the same as that of the maximum absorption of nuclein derivatives
bourow & Heyroth). The absorption spectrum of the cell-free filtrate of
killed by ultra-violet light or mechanically (Loofbourow, Cook, Dwyer &
) differs entirely from that of normal cells, and shows both active growth-
oting power for yeast and an absorption characteristic of cyclic nitro-
us bases (Sperti, Loofbourow & Dwyer). The same is true of breis of
hick embryo, irradiated and non-irradiated, tested on chick embryo heart
blasts in explant (Sperti, Loofbourow & Lane; Loofbourow, Cueto &
) and of mammalian embryos (Loofbourow, Dwyer & Lane). The active
r can be dried at 65° without injury (Loofbourow, Dwyer & Morgan),
contains guanine, adenine (not uracil), pentose (not desoxy-pentose) and
phorus (Loofbourow, Cook & Stimson). Since the substance is most amply

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n also in decarboxylation, for, as Baumann & Stare point out, thiamin pyrophosphate is
possibly linked with ribose and adenine in the cell, thus forming a nucleotide like the other
ymes.

given off by cells cultured in the most favourable medium, it is viewed rather as physiological response to injury than as a disintegration product of dead cel (Loofbourow & Dwyer). There can be little room for doubt that nucleotides a here again involved with growth-promoting power. Another indication pointi in the same direction is the curious phenomenon of "chromidial extrusion noted by many cytologists (Ludford; Horning & Richardson; Horning & Mille in tumour tissue. Chromatin appears in the cytoplasm and even in the inte cellular material; and the more malignant the neoplasm (i.e. the faster its growt the more pronounced the process is. Tumour tissue contains growth-promoti factor (Horning & Richardson). We shall shortly return to the significance cytoplasmic nucleotides (p. 636). Allied with this is the observation of not few cytologists that rapidly growing embryonic tissues frequently show pycnot nuclei and degenerating cells. In spite of the criticisms of Peter[2]; Glücksmann for example, seems to make good his case for a "*morphogenetische Degeneration.*" This might mean that the construction of nuclei sometimes normally overruns t amount of cytoplasmic nucleotide, and that the balance is redressed in this wa

A connection between irradiation products and animal tumours has no been made by Loofbourow, Cueto, Whelan & Lane. If minced rat or mous embryos are irradiated with lethal ultra-violet, and the cell suspension then free from débris by a Berkefeld filtration before subcutaneous injection into an adu rat, tumour-like masses arise. These are stated to consist of connective tissue an striated muscle. The detailed publication of this work will be awaited with interes

Some reference must be made here to the acceleratory action of embry extract on the healing of wounds, in favour of which there is a surprisin unanimous opinion (Carnot; Carnot & Terriss; Roulet; Wallich; Bergam Kiaer[2]; Morosov, Striganova & Skomorovskaia; and Schloss). Its action presumably due to the growth-promoting substance usually tested in other way The same acceleration has also been reported with A. Fischer's purified prepar tion (Nielsen; Waugh).

3·44. Nuclein metabolism

During the past ten years our knowledge of the nuclein and nucleoprote metabolism in embryonic development has made considerable advances, main centering round the existence of phyto-nucleic acid as well as thymo-nucleic ac in young growing tissues.

According to the classical descriptions phyto- or zymo-nucleic acid carried pentose as the central part of its nucleotides, while thymo- or animal-nucleic ac had a hexose instead. But the work of Levene, Mikeska & Mori established t fact that in both cases the sugar was a pentose, only in thymo-nucleic acid it a pentose with two OH groups (2-desoxy-*d*-ribose), while in zymo- or phyt nucleic acid it is a pentose with three (*d*-ribose)—see diagram.† In t ucleic acids four nucleotides are joined together, either in chain form or yclically, more probably the former, by their phosphoric acid groups. Further etails will be found in the monograph of Levene & Bass.

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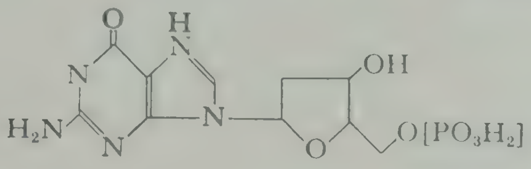
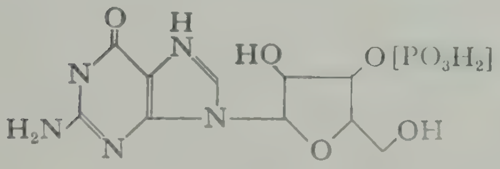
* So also T. K. Chang[2].

† The nomenclature here is now very unsatisfactory, for plant as well as animal tissues cont both kinds of nucleic acid. But perhaps phyto- and thymo- may be retained on account of brev as well as for historical reasons.



Guanine-2-desoxy-*d*-ribose nucleotide, typical of thymo-nucleic acid



Guanine-*d*-ribose nucleotide, typical of phyto-nucleic acid

The role of nucleins in embryonic development may best be approached by ay of the sea-urchin egg. Long ago Godlewski[1] and J. Loeb[2] observed an nmistakable increase in total nuclear material during its development from rtilised egg to free-swimming pluteus. Up to the blastula stage alone the increase of the order of 50 times. But as time went on a succession of workers (Masing[1,2]; hackell; Needham & Needham[2]; J. Brachet[5]) found that neither in nucleoprotein hosphorus nor in purine nitrogen is there any change during the development f the echinoderm egg. Nevertheless J. Brachet[1], who applied the Feulgen action, which is specific for thymo-nucleic acid, to the different stages of evelopment, found a consistent rise. He confirmed this further[2] by the Dische lorimetric method for thymo-nucleic acid, obtaining the following figures:

	Mgm./gm. dry wt.
Fertilised eggs	0·1
Gastrulae	6·2
Plutei	13·6

nere was thus a plain contradiction between the results of the various methods. was resolved by J. Brachet himself[3] when he estimated the pentose-content* the embryos, which was found to decline from 6·35 mgm./gm. dry wt. in the rtilised eggs to 2·6 mgm./gm. dry wt. in the plutei. The conception was thus veloped of an initial store of phyto-nucleic acid nucleoprotein which during velopment is gradually transformed into thymo-nucleic acid.† The former

Reference here and later in this section to estimations of "pentose" does not include the soxy-pentose of thymo-nucleic acid, which gives no furfurol under the same conditions.

And during maturation a converse process would have been going on (Brachet[23]). The conclu- ons of J. Brachet were questioned by two investigators. G. Schmidt[1] believed he could iso- te thymo-nucleic acid from unfertilised echinoderm eggs, but he relied only on the eulgen test, and after criticism from Brachet[9], later withdrew his claim[12]. The Feulgen test, in the sence of proper precautions, is given also by aldehydic phosphatides ("plasmal"), see p. 190; possible it should never be used *in vitro*. Hen egg-yolk, as was found by Marza & Marza[2], ves the plasmal Feulgen reaction, though it certainly contains no nuclein. Blanchard also lated small amounts of thymo-nucleic acid from unfertilised echinoderm eggs, but in this case s material was almost certainly contaminated by ovarian tissue.

must be in the cytoplasm;* the latter appears in the increasing total nuclea volume (Brachet[5]). Moreover, the undeveloped egg contains no free purine (Brachet[11]).

The question next arises as to whether this transformation† is a genera phenomenon. At present it is difficult to give a certain answer, but for marin invertebrates, at any rate, it seems fairly universal. The work of Needham & Needham[2] extended to the gephyrean worm *Urechis*, the sand-crab *Emerita*, an the brine-shrimp *Artemia*, as well as several species of echinoderms, and in al cases between 35 and 100 % of the eventual complement of nuclein phosphoru was to be found at the beginning of development. Henze significantly isolate 1 % of pentose from undeveloped octopus eggs. J. Brachet[11] found abundance o nucleoprotein phosphorus and pentoses in the eggs of the opisthobranchiat gastropod *Aplysia* and the spider-crab *Maia*. On the other hand, terrestria invertebrates seemed to occupy a different position, as the classical work o Tichomirov[1] on the silkworm had shown, by estimation of purine bases, th synthesis of 90 % of the nuclein present at hatching. But the newer figures Landi seem to show only a 31 % increase. Amboni finds about the same fo nuclein phosphorus, and Caspersson & Schultz[1], applying ultra-violet absorptio technique to the undeveloped *Drosophila* egg, find evidence of considerabl amounts of pyrimidine-containing nucleins. They also report strongly positiv qualitative tests for pentoses there.

The lower vertebrates occupy the same doubtful position. Large amounts o purine bases were isolated by earlier workers (Levene & Mandel; Tchernorutsky Steudel & Takahashi[1]) from teleostean eggs, e.g. cod, herring, carp, pike, etc. which would suggest that transformation rather than synthesis occurs durin their development. But v. d. Ghinst[2], working on the trout egg in J. Brachet' laboratory, found not only the expected appearance and rise of the materia yielding a positive Feulgen reaction, but also a clear rise of total pentose. Moreover, Rosenheim, C[illegible]šavičius, Ashford & Stickland, in preliminary experiments had noted a rise in nucleoprotein P in trout eggs during development (0 to 7 % of the total P); this was confirmed by v. d. Ghinst. A rise of pentose and nucleoprotein P was also found by J. Brachet[11] in the case of the dogfish, *Scyllium*. It i possible, therefore, that in fish eggs we have to deal with an initial store of purin and pyrimidine bases, followed by a synthesis both of phyto- and thymo-nuclei acid. As we shall see, the entire synthesis of all these compounds is carried ou by the chick embryo. Before passing to this, however, the amphibian embry must be mentioned. Again it seems to occupy an intermediate position, fo Plimmer & Kaya found only 28 % of the final nuclein phosphorus present at th beginning, while Graff & Barth recently reported an increase in purine nitroge in the frog egg beginning at early gastrulation and continuing with increasin speed till at the tadpole stage it had increased 100 % and they ceased to follow it ose confirmation of this is to be found in the figures of Takamatsu[1] on *Hynobius*. ıen v. d. Ghinst[1] and J. Brachet[13] found an increase in thymo-nucleic acid of ; mgm./1000 eggs (by the Dische method) and a decrease in pentose during e development of the frog, followed later by an increase.

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* This explains the early results of van Herwerden, who in 1912 described granules in echinoder egg cytoplasm which nuclease preparations digested like chromidia.

† Such a transformation is not very compatible with Just's otherwise ingenious attempt[3] to lin the appearance of nuclein compounds with the loss of morphogenetic potencies.

Events in the chick embryo, at least, are fairly clear, for it represents the other treme from the sea-urchin. Kossel's failure[1] in 1884 to isolate any purine base om the unincubated hen's egg was repeated by numerous later investigators ferences in *CE*, Section 10·1), who all demonstrated its synthesis by the ıbryo. Plimmer & Scott, moreover, found that 93 % of the nuclein phosphorus esent at the end of development was newly formed during the process. But endel & Leavenworth[2] also observed an increase of total pentose during development, and later this was explained by Calvery's isolation[1] of phyto-nucleoprotein om the chick embryo and his demonstration that this is synthesised as well as e thymo-nucleoprotein itself. The phyto-nucleoprotein of the chick embryo ntains guanine and adenine as purine bases, with cytosine and uracil as pyri-dines, instead of the thymine which the thymo-nucleic acid contains. Zinker-gel has lately established the existence of a thorough-going synthesis of purines the egg of the snake, *Tropidonotus natrix* (about 1 mgm. purine N per egg).

We may summarise therefore the ways in which nuclein metabolism may oceed, in four types of embryonic behaviour:

) Simple transformation from stored phyto-nucleic acid to thymo-nucleic acid	Echinoderms, worms, molluscs, arthropods
) Transformation, but synthesis of some thymo-nucleic acid	Amphibia.
) Store of purine and pyrimidine bases, followed by a synthesis of both forms of nucleic acid	Teleostean and selachian fishes.
) Complete synthesis of both phyto- and thymo-nucleic acid from non-cyclic precursors	Birds and reptiles.

e provisional nature of this classification, drawn from data which are, after still fragmentary, should not be forgotten. One should always remember that cleavage and pre-larval stages of the eggs of invertebrates are the most ıvenient for study, while in the higher organisms often only the later stages are ily had. There is nothing in the above table, therefore, inconsistent with the w that in *all* animals there is first a transformation from phyto- to thymo-cleotides, followed by a synthesis of both types of molecule. In any case, the ggestion earlier made by Needham & Needham[3], that only those eggs which ssess uricotelic metabolism can synthesise nuclein (cf. *CE*, p. 1158), must idently now be abandoned.

It may next be asked whether the nucleotides can be identified as intermediate oducts between phyto- and thymo-nucleic acid. J. Brachet[13] examined this estion in the eggs of the sea-urchin *Paracentrotus*, but there was no change at y stage in the relations between the mono-nucleotides and the protein-combined cleins. The former certainly do not disappear as thymo-nucleic acid is formed. this paper Brachet also showed by a balance-sheet that the phyto-nucleic acid disappears at every stage in quantitative correspondence with the thymo-nucle acid formed, both in the sea-urchin and in the frog. Because of Edlbacher's vie concerning the synthesis of the arginine-containing protein of nucleoprotein, examined echinoderm eggs both for arginase and nucleotidase (nucleopho phatase), but the results were negative. On the other hand nucleophosphata was found in amphibian eggs (I. Takahashi[2] on *Cryptobranchus*; Takamatsu[1] *Hynobius*).

As to the well-established fact that the chemical nucleoplasmatic ratio (*C* p. 1150) declines steadily during development, the above data throw no new lig on it. We have already seen it in Table 30, where nuclein P and purine N sho bradyauxesis. It is stated by v. Euler & Schmidt that tumour tissue shows t high nucleoprotein content characteristic of young embryonic tissue, but th requires further confirmation.

That phyto-nucleins occur in animal tissues is thus now far beyond doub Pancreas nucleoprotein, suspected as far back as 1894 to include nucleins "pentose" type, was proved to do so by Jorpes and Levene & Jorpes thirty-fi years later. The phyto-nucleins and nucleotides are becoming associated with th cytoplasm rather than the nucleus.* The work of Caspersson & Schultz† on th cytoplasm of *Drosophila* eggs has already been referred to, and these worke suggest that the cytoplasm of all growing tissues is characterised by ultra-viol absorption spectra which reveal the presence of the cyclic nitrogenous bases the nucleins. Apart from plant examples, they find[2] that the imaginal discs the *Drosophila* larva show a strongly absorbing cytoplasm, while the mature larv gut cells show no trace of the peak. They further suggest that the cytoplasm phyto-nucleins are responsible for the well-known histological basophily embryonic tissues.‡ In this connection it is interesting that the first nucleoti of phyto-nuclein type to be isolated from animal tissues, inosinic acid, hypoxanthine-*d*-ribose nucleotide (Liebig in 1847), originates, as is now know directly from the adenylpyrophosphate which is one of the coenzymes of glycolys (see p. 209). Moreover, in many if not in all developing eggs adenylpyrophospha is synthesised (cf. pp. 617 ff.). Enzymes which may be responsible have bee studied by v. Euler & Skarżyński.

All this goes to prove the essential nature of the phyto-nucleins for embryon development, and to it we must add Claude's isolation[1] of phyto-nuclein materi similar to that of the Rous sarcoma factor from chick embryos (see p. 269), an Fischer's identification[7] of phyto-nucleins in the growth-promoting factor chick embryo extract (p. 630). If nothing has been said in this section about th role of nucleins as the carriers of genetic influences in the nucleus, it is on because as yet this, perhaps their most important function, has not been broug into direct chemical relation with embryonic metabolism. But it is only necessa to glance through Sections 2·64 and 2·67 to see how direct this relation w probably become and how important for morphogenesis itself. In the fact th h viruses and genes are self-perpetuating and that both are nucleoproteins we ! that we have found an essential key to the understanding of the reproduction living organisms in all its aspects. And for this reason it is significant that mo-nucleic acid is a highly anisometric micelle or molecule (300 times as long it is thick) with great optical anisotropy — a fibrous macro-molecule (Signer, spersson & Hammarsten; Greenstein & Jenrette). It is believed that poly- ›tide chains run lengthwise along the chromosome, with their basic arginine e-chains united with the phosphoric acid groups of the nucleotides (Astbury[2]) ich have exactly the same spacing. But this is as much as there is room to here on this subject, which may be followed in reviews such as those of :bury[1] and Astbury & Bell. We need only add the curious observation of these rkers, interesting in view of what has been said above, that phyto-nucleic acid :s not give optically anisotropic solutions nor a fibrous product on drying. we could have a full knowledge of the physico-chemical properties of the › nucleins, the whole contents of this section would probably fall into a rational ler..

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* Though the nucleolus is said to be ribo-nuclein (Caspersson & Schultz[3]).

† Description of methods in a paper by Caspersson.

‡ Cf. the remarks above (p. 632) on chromidial extrusion in rapidly growing neoplasms.

3·45. Lipin and sterol metabolism

Γhe earlier work on the lipin and sterol metabolism of the hen's egg during relopment, summarised in *CE* (Sections 12·1 and 12·4), led, apart from ferences in detail, to three rather simple conclusions. In the first place the al fat of the egg decreases, on account of the considerable fat combustion, inly during the last week of incubation, with a total loss of some 55 % of the ginal store by the time of hatching (*CE*, Fig. 359). Secondly, the phosphatides :sent in the yolk at the beginning also suffer a regular fall, similar in its time :idence and of about the same relative magnitude (*CE*, Figs. 373 and 374). the same time there occurs a correspondingly large increase of inorganic osphorus (deposited in the new bones) and a lesser increase (as already men- ›ed) of nuclein phosphorus. To these phosphorus is also contributed from the ıkdown of the phosphoprotein (cf. p. 15) in the yolk at the beginning of elopment. Alone of all the phosphorus fractions the water-soluble organic ws little change. Thirdly, in contrast to the great utilisation of neutral fat and ›sphatides, the total sterol, mostly cholesterol, shows practically no change ›ughout incubation (*CE*, Fig. 389), although there is a clearly marked increase :holesterol esters and decrease of free cholesterol.

'Vith these well-established facts as background, we may see how the newer thods of approach have further illuminated the processes going on. The ıeral disappearance of neutral fat has been confirmed by a number of investi- ors, e.g. Romanov[5]; Kusui[1]; Jost & Sorg, though as they did not always follow ›ubation through till quite the end, their figures for total fat combusted are newhat lower than that given in Table 10. Jost & Sorg paid special attention the parallelism between loss of fat and loss of phosphatide. They found that the 11th day 6·3 % of fat and 5·04 % of phosphatides had been lost, while on 19th day the corresponding figures were 42·1 and 42·5 %. This they inter- ›ted as favouring the view, for which much other evidence has been brought forward, that fatty acid glycerides must be combined with phosphoric acid bef they can be oxidised. Measurements of iodine values of the fat and phosphatid of the whole egg and the embryo and yolk separately made by Kusui as well Jost & Sorg are in agreement with such a view. Thus throughout developme the iodine value of the neutral fat remains steady at 75–76, while that of t phosphatides sinks from about 71 to 55. Moreover, this latter process occurs a greater extent in the yolk than in the embryo, and all workers agree that t fat of the embryo is more unsaturated than that of the yolk. It may be allowab therefore, to picture a preferential absorption of unsaturated fatty acids into t embryo, and a preferential combustion of those phosphatide molecules containi unsaturated fatty acids. This reinforces the impression gained from the earli work (*CE*, p. 1171). Kusui adds the interesting facts that free fatty acids in yo reach a peak on the 3rd day of incubation (cf. the lactic acid peak about the sam time, already referred to, p. 608) at 3 % of the total, while in the embryo itse they are maximally present on the 14th day at 9·7 % of the total. Volatile fat acids are never present in any part of the egg in more than traces.

An observation of Romanov's[9] the significance of which is not entirely clear that while the refractive index of the neutral fat from the yolk remains consta at 1·4690 throughout incubation, that of the embryo fat declines very regular from 1·4890 at the 9th day to 1·4720 at hatching. This is quite in agreement wit the more unsaturated nature of the embryo fat, but it may also mean that th fatty acids of the embryo have relatively longer carbon chains than those of th yolk.

Just as Jost & Sorg investigated the relations between the quantities of neutr fat and phosphatide, so Kugler examined the relation between the two mos important substances constituting the group of phosphatides, lecithin an kephalin. As noted already from Table 30 phosphatides are built into the bod of the embryo bradyauxetically with a *k* of about 0·75. But during the process according to Kugler, a rather strict ratio of 3 of lecithin to 1 of kephalin observed, both in the waning yolk and in the waxing embryo. Whether this rati obtains in all birds' eggs throughout development seems doubtful, if the figure of Masuda & Hori are trustworthy.

The question now arises as to whether the neutral fats, phosphatides and sterol of the yolk are transferred as such into the embryo or are first broken down t simpler molecules and then rebuilt. In recent years it has been possible by th use of molecules labelled with isotope elements to gain answers — at any rat provisionally convincing — to all three of these questions (see the review Hevesy). Taking first the phosphatides, Hevesy, Levi & Rebbe injected radio active sodium phosphate into hen's eggs before incubation for varying periods time. It was found that the phosphatide phosphorus extracted from the embry showed a high activity while the phosphatide phosphorus extracted from the yol showed none. Phosphatide molecules present in the embryo must therefore hav been synthesised and not taken over directly from the store in the yolk; th constitutes strong support for the "carrier" theory represented by Jost & Sor namely that combination of fatty acids with phosphorus assists their oxidatio e it shows that a complete breakdown does intervene between yolk and embryo sphatide. It may also be mentioned here that the acid-soluble phosphorus the nucleoprotein phosphorus also showed much radioactivity, thus demonting that they also had arisen by a synthesis for which inorganic phosphate been employed. Similar results were obtained when labelled hexosemonosphate was introduced into the embryo at the beginning of incubation, wing that it had been broken down to inorganic phosphate and that this had n been used for the various syntheses.

n sharp contrast with these findings were those of workers who introduced vy hydrogen isotope into the egg at the beginning of incubation (see the iew of Schoenheimer[3]). After the injection of a sufficient amount of heavy er into the egg the amount of deuterium in the fatty acids of the chick embryo ards the time of hatching was measured (Schoenheimer & Rittenberg[1]). It was nd that the fatty acids had taken up no deuterium whatever, in contrast to e synthesising fatty acids on a carbohydrate-rich diet, where the fatty acids idly take up deuterium. The conclusion was therefore reached that fatty acids simply transferred as such from yolk to embryo and that no intermediate akdown occurs.* Exactly the same result was obtained for the cholesterol of embryo by Rittenberg & Schoenheimer, again in contrast to the entry of iterium into the cholesterol of adult mice.

Ve must therefore picture the fat and cholesterol stores of the embryo being nposed of molecules taken directly from the yolk, while the embryo's phositide molecules are new. Neutral fat and phosphatide in the whole egg both atly decrease, the latter probably acting as a carrier for fatty acids destined for nbustion, and itself supplying phosphorus for many synthetic purposes, inding the provision of phosphatides for the embryonic tissues.

The general conclusion that no synthesis or destruction of cholesterol occurs ing the development of the chick embryo is supported by the work of rżynski[2] and Serono, Montezemolo & Balboni, though like so many other stigators (see *CE*, pp. 1222 ff.) they got fairly consistently the slight rise, in rżynski's experiments an average of 9·5 %. That this is significant has never n demonstrated. A new observation was the definite decrease in ergosterol tent (from 0·23 to 0·12 % total sterol) and the absence of any change in the ydrocholesterol content. It is thought by these authors that the sex-hormones formed from cholesterol, but this still awaits a definite proof. According to oulleau the fertilised hen's egg has 2γ gm. oestrone, and it is certainly thesised fairly steadily by the embryo (Serono, Montezemolo & Balboni); p. 316. Dalton[3] has again studied the appearance of the anisotropic droplets cholesterol esters (*CE*, p. 1219) in the chick embryo liver; according to him y appear normally on the 11th day of incubation, but their appearance may hastened considerably by grafting the liver on the chorio-allantois of older bryos. The blood cholesterol of the chick embryo, measured by Zorn &

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his is substantially, not absolutely, true, because in later experiments of Schoenheimer's, re the embryo was carefully separated from the spare yolk at the end of development, a minute unt of deuterium was found in the fatty acids, indicating very slight synthetic processes.

Dalton, rises to a maximum of double the adult value at the 18th day of incul tion, but returns nearly to normal at hatching. Infiltration of the liver w neutral fat begins somewhat before, about the 15th day, in Trotti's descriptio At this time liver lipase is increasing in activity (Galvialo & Goryukhina), wh the lipase and tributyrinase activity of the whole embryo increase stead throughout (Ammon & Schütte; Kolobkova — see Fig. 72).

A view of the chick's fat metabolism at hatching has been given by Entenmar Lorenz & Chaikov. The bird hatches with marked lipaemia and a "fatty live containing enormous amounts of cholesterol esters. After hatching, the lipi and sterols of liver and blood return fairly quickly to normal levels.

To the above account need only be added a mention of numerous histochemic papers on the appearance of fat in embryonic organs and in explanted pieces them, to which reference must be made by those interested (Konopacka[1] Zweibaum; Szantroch; Rix; Haszler; Hadjioloff; Hadjioloff & Usunoff).

Nothing comparable with the above-described advances has been made in t case of the mammalian embryo, which almost certainly derives the greater pa of its fat from the maternal blood stream, and whose relative fat combustion h so far proved impossible to measure. Interest has mainly centred round t supply of neutral fat; and the plan of altering the degree of unsaturation of t fat in the maternal diet, and then examining whether the foetal fat has chang in the same direction and to the same extent as the maternal fat, has been tri again and again. The previous conclusion, that the foetal fat *can* be changed this way, but not so readily as the maternal fat, remains unaltered. Differe workers have had different degrees of success. Sinclair[2]* found that after co liver oil feeding, the iodine values of the neutral fat and the phosphatides of r foetuses were significantly raised, the former nearly 100 % and the latter 25 % Similar results were reported for rabbits by Bickenbach & Rupp[2]. Continui Sinclair's work on rats, Chaikov & Robinson found the same variation in t foetus, but noted that it was distinctly less than that in the mother, which reach 400 %. Substantially the same conclusions were reached by Miura[2], who al found that elaidic acid can be incorporated into the phosphatides of the foetu With this McConnell & Sinclair agree, but point out that the transfer of elaid acid appears to take place much more effectively by way of the milk than throu the placenta.

The characteristics of the fat and phosphatides normally present in the mar malian foetus at different stages have been studied by many workers (Torrisi the dog; Chaves on the hedgehog; Martinoli and Roussel & Deflandre[2] on t cow and sheep; J. Suzuki on the rabbit; Lovern[1] on the porpoise; and Cattaneo man). The details, as yet fragmentary, hardly lend themselves to any generalis tion, and must be found in the original papers.

According to Schoenheimer & v. Behring the meconium of the human foet contains surprisingly large quantities of cholesterol; and cetyl alcohol, a substan (see p. 249) which has been suggested by Schoenheimer & Hilgetag to be a natur endogenous purgative, has been found in meconium by Schoenheimer[2]. Anoth isolated point of interest is that curious tissue, Wharton's jelly, of the umbilic

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* Sinclair attributes to me the view that the mammalian placenta is impermeable to fat and hen that the foetus must synthesise all it contains from other substances. Reference to *CE*, pp. 11 and 1524, will show that I never put forward any such suggestion.

, which has been found to be almost devoid of fat, phosphatide, or sterols d[2]; Mangili).

ievitz & Hevesy have found that radioactive phosphorus is taken up by the oetus and the human placenta, probably in the synthesis of phosphatides as we have seen to occur in the chick embryo.

e question of possible changes in the protein carriers of enzymes concerned fat metabolism was examined by Bamann, Mahdihassan & Laeverenz, with tive results, for they found no changes between the 6-month human foetus he adult in the optical specificity of liver esterase, tested on racemic mandelic ethyl ester. This is in contrast with catalase (p. 604) and haemoglobin 00), which do change.

e lipin metabolism of the eggs of lower vertebrates has unfortunately ved little study. Working on those of the trout, S. Smith[1] found that the entage of fat in the yolk-sac rises from 10 to 30 %, either because of a nesis of fat or more probably because of preferential protein utilisation, up e 80th day of development. Over the whole period there is a definite loss of rom the system, as shown in Table 11, analogous to the hen's egg. Protein bustion predominates from the 40th day and goes on till the 70th; fat bustion begins at the 56th day and lasts till the end of the yolk-sac period. n the last of the stored reserves are used, the alevin lives on its muscle ein. A similar study was made by Hayes & Ross on the eggs of the salmon. the fat loss in percentage of the fat originally present was found to be high, %. These authors believe a synthesis of fat occurs in the earlier half of lopment, but their figures do not clearly show this. They also believe that naximal period of fat absorption corresponds with the maximal period of fat bustion, contrary to what is the case in the chick embryo (see *CE*, Fig. 251); may well be so. Whether it is correct to identify the peak in fat-absorption shown by the salmon 30 days after hatching with the peak on the 9th day of ation in the chick seems doubtful, though Hayes & Ross bring forward sting morphological comparisons suggesting that 10 days of salmon embryo approximates closely to 1 day chick embryo time. Masuda & Hori state he lecithin-kephalin ratio differs a good deal in fish eggs from its avian value.

at a synthesis, and hence a total overall increase, of total fat can occur in yonic development is an old observation in chemical embryology (see *CE*, 88) and has been noted in a urodele amphibian, teleostean fishes, and ially pulmonate gastropods (cf. p. 576, where the effect of this on the R.Q. nentioned). The urodele which had shown it was the American salamander *tobranchus alleghaniensis*, but this has not been confirmed on the Japanese es, *japonicus*, where Tomita & Fujiwara and Kataoka & Takahashi find a net fall of total fat, as noted in Table 11. Moreover, the two pieces of work e mentioned did not show striking evidence of a synthesis in teleostean eggs. subject therefore remains obscure.

all amphibian eggs there is certainly a combustion of fat (see *CE*, p. 1173) Takamatsu's figures[2] on *Hynobius* illustrate it again. The work of Kataoka akahashi on the salamander during development showed that no change occurs in the iodine value of the fat, and that of Iseki & Kumon indicates th half the phosphatide is broken down, as in the hen's egg. There seems to be slight fall in the total cholesterol, and it is interesting that there is an increa in cholesterol esters and a decrease of free cholesterol, just as in the chick embry Differences in the cholesterol content of tadpoles raised in iodine-containing ar iodine-free waters have been reported by Brambel & Lynn; these require co firmation. Tributyrinase was found by Takamatsu[1] in *Hynobius*, but not I. Takahashi[2] in *Cryptobranchus*, embryos.

Invertebrates also, as would be expected, for the most part show considerab fat combustions. Pelluet counted the fat globules in the blastomeres of echin derm eggs and found that their number steadily decreases from unfertilised eg to blastula (from 14 to 10·75 granules/cu.μ). The loss of fat was confirmed ar substantiated chemically by Hayes[3], who found the total loss up to 40 hr. (th pluteus stage) in the case of *Arbacia* to be 42·5 % of the initial fat store, a figu higher than that previously current (see Table 11). Hayes represents his da by a curve which descends to a minimum, then rises again and finally falls, bu it seems more likely that a general downward trend is all that one would b justified in asserting, since much depends on a single high point at 0 hr. and single low one at 43 hr. In consequence of this, his rather elaborate argumen directed to showing that the hitherto unidentified acid produced after fertilisatio (see p. 572) is really a fatty acid, has little force. Interesting, in view of fac already mentioned with regard to the chick and amphibia, is Hayes' finding th during the development of *Arbacia* no change occurs in the total cholesterol.

In insect eggs it has several times been shown that events take place in a wa very similar to the hen's egg as far as fat (e.g. Busnel on the potato beet *Leptinotarsa*) and phosphatide phosphorus is concerned. Inorganic and nucle protein phosphorus increase at the expense of phosphatide and phosphoprote phosphorus (Akao[2] and better Amboni on the silkworm moth *Bombyx*; Thompso & Bodine[2] on the grasshopper, *Melanoplus*). But while Akao (whose results i general are, it must be admitted, erratic and puzzling) found only a slight increa of total cholesterol, Murao, in a more convincing paper, found regular an persistent increases of some 30 % in total sterol during the silkworm's develo ment. Should this be confirmed, it will constitute an interesting exception the rule that in general sterols are not synthesised by embryos. It may b significant in this connection that a special form of sterol, bombicesterol, occu in the eggs of the silkworm moth.

3·455. Choline esterase and the development of nervous function.

Since choline is the nitrogenous base in lecithin, a few words must be said i this section on the subject of choline-esterase. As we have already seen (p. 211 acetylcholine is given off from nerve-endings of the "cholinergic" type, probab by sudden liberation from a bound form. It may perhaps have special si nificance also in the placenta (p. 86). But those who have studied cholin esterase, the enzyme which restores the *status quo* by destroying the stimulatin ıpound, have not neglected to examine the appearance of this enzyme during bryonic development.

n the brain, of the chick for example, it rises steadily during the incubation iod (Nachmansohn[1]) from Q_{ChE} 1·3 to 20·8, reaching the adult level a few days r. On the other hand, in striated muscle the Q_{ChE} rises to a level much higher ·0) than that characteristic of adult muscle, after which it falls to the adult l. The peak is reached by the chick embryo about the 18th day of develop- nt (Nachmansohn[2]) and by the rabbit about the 7th day of post-natal life ibson). It is explicable by the relative proportions of motor nerve-endings muscle fibres in the developing muscle. Choline-esterase is localised round former (Marnay & Nachmansohn; Couteaux & Nachmansohn; T. P. Fêng . C. Ting). Hence if the nerve-endings multiply at first more than the muscle es, a peak in choline-esterase would be expected. Cardiac muscle, however, aves more like brain, in that no peak is seen, the choline-esterase content dily rising to the adult level (reached at the 10th day of post-natal life in the bit; Shamarina). In tissue cultures of embryonic heart muscle, nerve fibres endings soon disappear; choline-esterase disappears too (Thomas & Nach- nsohn). Other aneural but enzymatically very active tissues, such as the yolk- oderm and *area vasculosa* ectoderm of the chick, examined by these authors, e, as would be expected, extremely low values for Q_{ChE}.

achmansohn[3] summarises all these results in the statement that choline- rase increases during development *pari passu* with synapses and motor nerve- ings. This is borne out by independent investigations of Rjabinovskaia[2] and essunin, who found that during development the properties of embryo brain acts change from adrenalin-like to acetylcholine-like. One fact of real interest hat, according to Youngstrom, choline-esterase activity in amphibia reaches nsiderable value before the differentiation of the central nervous system has e far and before movements have been initiated.* Estimations of acetyl- ne are reported for the whole chick embryo by Z. Y. Kuo[7]. At 2·5 days' ıation the embryo contains about 0·33 γ/gm. and by 5 days this has risen to γ/gm., after which the amount remains constant till at least the 12th day.

46. Pigment metabolism

nong the synthetic powers of developing embryos none is more striking than laboration of pigments which they perform. The amounts of pigment in the ıbranes and jellies of eggs are relatively small; and the egg-cell itself, or in er forms, the yolk, contains nothing but carotenoids which are, so far as we

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is felt that the literature on the initiation of movements, reflexes, and the like, is too physio- al to be reviewed in the present book. Recourse must be had to the monograph of Coghill the numerous papers of Windle; Z. Y. Kuo; L. Carmichael, and their colleagues. Here it must e to mention the important fact that muscular movements and nervous co-ordination arise elf- rather than functional differentiation. For if amphibian embryos are allowed to develop aesthetic solutions which maintain them immobile, they will manifest, upon being returned sh-water, all the performances which normal animals show at the same stage of differentiation thews & Detwiler; L. Carmichael). Recent work has not given much support to the old that myelinisation of the central nervous system is a limiting factor in the development of ements.

know, unessential to the life and morphogenesis of the new individual. Th synthesis of haemoglobin is, it has been said, the most spectacular of all th syntheses. And although we certainly have more light than we had ten years ago its mechanism still remains very obscure.

This section will divide itself naturally into (*a*) the pyrrol pigments (comprising both porphyrin metabolism and iron and copper metabolism), (*b*) the carotenoid pigments, (*c*) the flavines, (*d*) the pterines, and (*e*) melanin.

3·461. Pyrrol derivatives

If we glance at a scheme of the formulae of the porphyrins we can see tha owing to the great complexity of these tetrapyrrol compounds, there are a larg number of possible variations, differing in the symmetry of the positions at which the side-chains are attached.* Yet out of the various theoretical possibilities only those porphyrins of types I and III occur in nature. It is not believed that they can be converted one into the other without breakdown. The aetioporphyrin have as side-chains methyl and ethyl groups, the coproporphyrins have methy and propionic acid side-chains, the uroporphyrins have acetic acid and propionic acid side-chains, and protoporphyrin has methyl, vinyl and propionic acid side-chains. As shown in the diagram chlorophyll is derived from aetioporphyrin III and the haem of haemoglobin from protoporphyrin III. In chlorophyll the pyrrol nitrogen atoms are linked by a magnesium atom; in haemoglobin they are linked by an iron atom, and in turacin, a red pigment from the feathers of a family of tropical birds,† the pyrrol nitrogen atoms of uroporphyrin I are linked by a copper atom. Uroporphyrin I also occurs in pathological conditions, such as ochronosis, accumulating in the bones.

The physiology of the porphyrins of the type I series is still, indeed, to some extent a mystery. Coproporphyrin I seems to have no functional role in the adult,‡ but the impression is growing that just as the bile pigments are stages in the breakdown of haemoglobin, so the porphyrins of series I are by-products in the synthesis of those of series III. Thus the former are always found to appear whenever an intensive haemopoiesis is going on,§ and as we shal see, this evidence is reinforced by what happens during the development o the chick embryo.

A word must first be said, however, about the porphyrin which is deposited in the shell of the hen's egg. A number of workers had observed that egg-shells give a red fluorescence in the Wood's (ultra-violet) light (Derrien; Tapernoux Bierry & Gouzon; Gouzon; Dhéré), and this was shown to be due to a porphyrin rst called ooporphyrin, but later identified by Fischer & Kögl and Fischer indner as protoporphyrin III. On very long keeping, the fluorescence goes partly into the egg-white (Baetsle) and then disappears altogether (Straub, Stijgeren & Kabos). There is said to be a trace of porphyrin in the shell-

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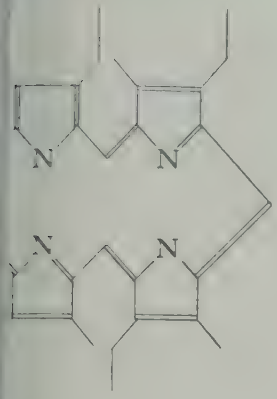
* For porphyrin nomenclature see Fischer & Stangler, and for porphyrin metabolism in general the monograph of Carrié and the reviews of Lemberg^{3,4}.

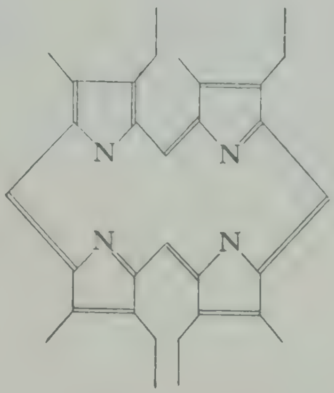
† This pigment is water-soluble. It is worthy of being put on record that Sir F. G. Hopkin had some of these feathers, which he used to shake about in water before his class. Anyone wh was not fascinated by the beautiful red pigment which dissolved out would, he said, never mak a biochemist.

‡ But it is the principal porphyrin synthesised by yeast (Carrié & Mallinkrodt-Haupt).

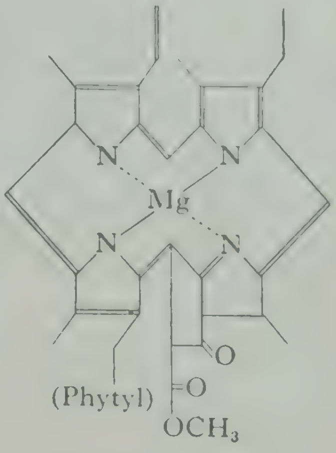
§ And even in normal adult life, very small amounts of coproporphyrin I are constantly excrete (Dobriner).



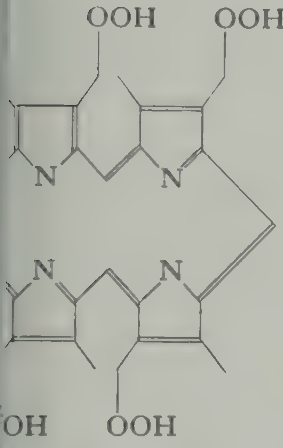
etioporphyrin I



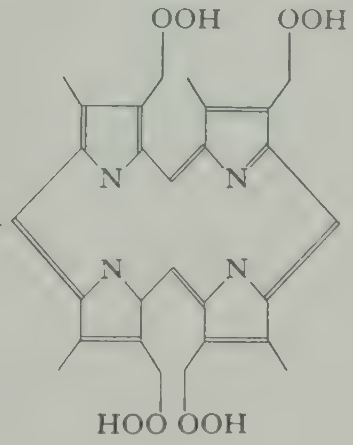
Aetioporphyrin III


N
N
Mg
N
N
O
(Phytyl)
O
OCH₃

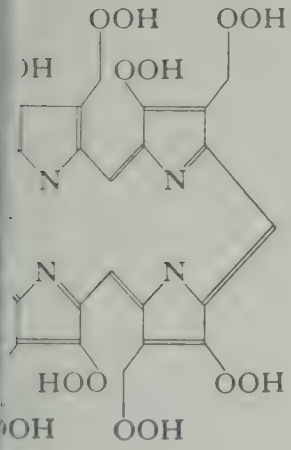
Chlorophyll α


OOH
OOH
N
N
N
N
OH
OOH

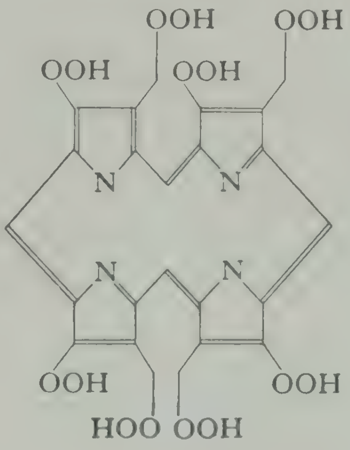
oproporphyrin I


OOH
OOH
N
N
N
N
HOO
OOH

Coproporphyrin III


OOH
OOH
H
OOH
N
N
N
N
HOO
OOH
OH
OOH

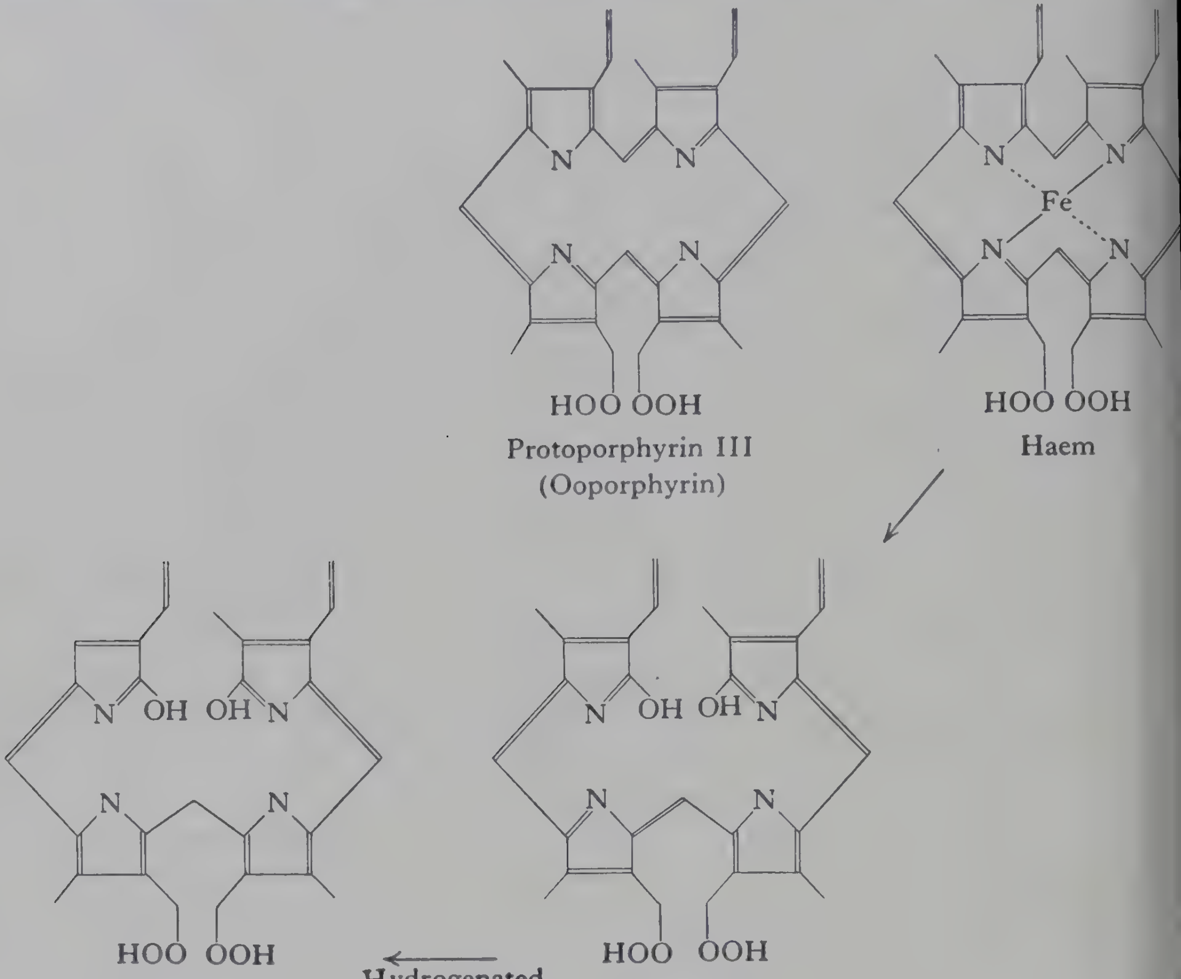
Uroporphyrin I


OOH
OOH
OOH
OOH
N
N
N
N
OOH
OOH
HOO
OOH

Uroporphyrin III

membrane (Klose & Almquist). On repeating Buckner's experiment* w opened egg-shells, van den Bergh & Grotepass found that no porphyrin lea the shell within a few weeks, but nevertheless within the first week of develo ment there is an increase of from 4 to 140γ of porphyrin per egg, and so of this is in the yolk and white as well as the embryo.

The subject was gone into more thoroughly by Schønheyder, who establish the fact that *both* coproporphyrin I and haemoglobin (i.e. protoporphyrin I are synthesised during the development of the chick embryo. The form



Protoporphyrin III
(Ooporphyrin)

Haem

Bilirubin

Biliverdin
(Uteroverdin, Oocyan)

increases from a trace to $7{\cdot}5\gamma$; the latter from a trace to about 150 mgm. Wh the rates of increase are plotted heterauxetically it is found that coproporphyrir is synthesised much more slowly than the dry weight of the body (k only 0·3 while haemoglobin is made more rapidly ($k = 1{\cdot}11$), till at the end of incubatio there is about 20,000 times as much haemoglobin as coproporphyrin I.† Fro the fact that bile pigment does not appear before the 7th day (Sendju) whi coproporphyrin I has considerably increased by then, Schønheyder argues th not likely to have been formed by the breakdown of red blood corpuscles. coproporphyrin III was found in any of the fractions, and there was no roporphyrin I in the shell.

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* This consists in opening an egg, removing the contents, and bubbling CO_2 through a quant of water placed in it (*CE*, p. 1268).

† Were it not for the great disproportion between the amounts of porphyrins of the two ser formed, it would be tempting to analogise this dual synthesis with the formation of *both* kinds nucleotide (p. 635).

n mammalian embryos, especially those of rodents, it has often been observed the bones are rich in a red pigment, and this is known to be either uro- or ro-porphyrin (Derrien; Fikentscher[1]; Borst & Konigsdorfer). After the end oetal life the colour in the bones fades away. This fits in with a number of s. Thus there is porphyrin (either coproporphyrin I or III, not protopor-rin) in human foetal blood, according to Fikentscher[2], but never in maternal od nor normal adult blood. The placenta is reported to be impermeable to phyrins (E. Fraenkel). Porphyrin is found in small amounts, about 5γ %, uman amniotic liquid (Fikentscher[1]), and (an old observation) in the meconium Gunther; Stockvis), where it has been identified as coproporphyrin I by ldenström. Herold finds that it is excreted by the new-born human infant ching a maximum of 8γ per day on the 4th day of extra-uterine life. Every-ng points to a physiological porphyria in foetal life, probably not unconnected n the vigorous haemoglobin synthesis then going on.

Ience the observations of W. J. Turner acquire much interest. The Penn-anian fox-squirrel, *Sciurus niger*,* has long been known to have bright red es, and Turner was able to identify the pigment as uroporphyrin I. He ciated the formation of the pigment with the megaloblasts of the bone-row, and suggests that in this animal we have an exceptional persistence into lt life of what is the general normal way of haemoglobin synthesis in foetal .

ince there are none of the cytochromes nor much catalase in the undeveloped 's egg, it is obvious that these haem compounds must also be synthesised the developing embryo. Unfortunately, no exact studies have been made of ochrome synthesis (that of Ito is purely qualitative). Catalase synthesis is rred to elsewhere (p. 604).

urning now to the bile pigments, it will be seen from the formulae that they ist of four pyrrol nuclei no longer united in a ring. Bilirubin is the hydro-ted form of biliverdin, which may arise from haem by way of the inter-iate compound verdo-haemochromogen, where the tetrapyrrol chain is ned but the iron atom still retained (see the discussion of Lemberg[3]).

iliverdin is generally found wherever intensive breakdown of haemoglobin ing down. It was described by Lemberg & Barcroft (under the name of overdin; Lemberg, Barcroft & Keilin) in the characteristic green regions of dog's placenta, where it occurs in such quantities as almost to equal the total unt of haemoglobin in the body of the foetal dog. Uteroverdin was later tified with biliverdin by Lemberg[2]. Less easy to account for is its presence he egg-shells of birds, from which it was isolated by Lemberg[1] (under the e of oocyan). The blue colour of the eggs of many species of birds, such as gulls, is due to this pigment, and Punnett has found that its deposition in the

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ne specific name of this animal is perhaps no coincidence. If it were not heavily pigmented ould probably suffer from photosensitivity, as human porphyrinurics do.

egg-shell occurs also in certain races of domestic fowls, among which it see to have arisen as a dominant mutation in South America. In some egg-shell such as that of the cassowary, oocyan, or some pigment extremely like it, present in combination with protein, according to Dinelli.

In mammalian embryos bile-pigment production may play a more importar part, as the well-known jaundice of the new-born human infant (*icterus neonatorum*) shows. As we have already seen (p. 598) the mammalian foetus, toward the end of its intra-uterine life, enters into a condition of anoxaemia similar t that experienced at high altitudes. To this it reacts, as Anselmino & Hoffmann have pointed out, in several ways; the pulse rate increases,* the heart hypertrophies, the haemoglobin content of the blood, the red blood corpuscle count an the blood volume all rise, and there is a shift in the dissociation curve of th haemoglobin. All these processes occur to more or less the same extent i acclimatisation to high altitudes. That the haemoglobin and the erythrocyte increase appears from many studies. In the rat, for example, the haemoglobi content rises from 5·5 to 10·78 gm./100 c.c., and the red blood corpuscles fron 694,000 to 2,600,000/c.mm. In man, the haemoglobin content reaches 22·5 gm. 100 c.c. blood, higher than at any subsequent time. Now after birth this loa of haemoglobin becomes excessive and is rapidly got rid of. What happens to th iron is uncertain; some believe that it is stored in the infant's liver (Gladstone Ramage, Sheldon & Sheldon), others think that it is lost from the body (Stearns Stearns & Singer; Stearns & McKinley). But the porphyrin undoubtedly break down, for Yllpö discovered a physiological post-natal bilirubinaemia, an Anselmino & Hoffmann[2]† put forward the plausible view that if the destructio of porphyrin goes on faster than the bile pigments can be excreted, *icteru neonatorum* will follow. At the same time it must not be supposed that n breakdown of haemoglobin is going on before birth; on the contrary bile pigment are always accumulating to a certain extent, and the breakdown process can eve be carried out, according to Sümegi & Csaba, by embryonic cells cultured *i vitro*.

All workers agree, as we have seen, that during embryonic life, the haemoglobin content of the blood and the erythrocyte count increase steadily up to th normal adult level, and then greatly overpass it, doubtless as an adaptation to th relatively anoxaemic conditions of late foetal life (cf. p. 600), falling graduall to normal after birth. This occurs not only in mammals (as witness Kindred & Corey and Nicholas & Bosworth on the rat; Zeidberg and Kunde, Green Changnon & Clark on the rabbit; Jones, Shipp & Gonder on the pig; an

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* Usually foetal heart rate exceeds maternal heart rate; cf. the following figures from Hartman Squier & Tinklepaugh (beats/min.):

	Foetal	Maternal
Man	135	70
Cow	161	50
Dog	150	100

And in the chick, as Bogue has shown, the rate increases from 130 to 235, the high level bein attained by the 10th day, i.e. before the respiratory difficulties become acute.

† Some of their views were contested by Haselhorst & Stromberger[2] but the main idea holds goo (Anselmino & Hoffmann[3]).

ıtrobe & Shumacker[2] on many mammals especially thoroughly), but also in chick, where, as Sümegi and Zorn & Dalton show,* the adult level, both as ırds haemoglobin content and erythrocyte count, is passed about the 12th day ncubation, and by the time of hatching almost doubled. It has also been ıonstrated, by Holmes, Pigott & Campbell, that the chick shows the great rease in blood haemoglobin following hatching, just as mammals do following h.† In all these forms, as Sabin, Miller, Smithburn, Thomas & Hummel shown, the approach to adult leucocyte content is very much slower, adult l not being reached in the rabbit, for instance, until the end of the 6th month xtra-uterine life. This enhances the plausibility of the view that the high noglobin content is a direct adaptation to the embryo's respiratory difficies.‡

ut now Wintrobe & Shumacker, in their elaborate studies just referred to, erved that as the number of red blood cells in the circulation increases, their ividual diameter and volume greatly decrease. Thus in the pig the number ed blood cells climbs from 0·2 million/c.mm. in early foetal life to 3·6 million/ m., but during this time the average diameter of an erythrocyte has fallen n 16μ to 7μ, and its average volume from 360 cu.μ to 70 cu.μ. In other words, foetus, as they expressed it[1], exhibits in the earlier stages a hyperchromic rocytic anaemia, accompanied by the presence of many immature erythros. And they pointed out that this condition resembles so exactly the blood ure in patients suffering from pernicious anaemia that it is difficult to inguish the slides under the microscope. Curves showing the return to mal after the beginning of liver treatment may also be superimposed upon se drawn against foetal age. They suggest, therefore, that perhaps the haematotic tissues of the foetus are being influenced by the anti-anaemia principle he liver, as part of the physiology of normal development.

s is generally known, the work of Castle, Townsend & Heath showed that nti-anaemia principle of adult mammalian liver is formed from two sources, trinsic factor in the diet, and an intrinsic factor provided by the wall of the ach.§ For the foetus the principle can hardly come through the placenta t & Hays), though it has been found there by Minot. Should the drain of foetus be too great, pernicious anaemia, either of the maternal organism uss) or of the new-born (L. G. Parsons), may develop. The principle has found in foetal liver by Wintrobe, Clark, Trager & Danziger, towards the of pregnancy.

xtremely little is yet known about the chemical nature, either of the principle he two factors. It seems very likely, however, that the pterines (complex nes; see below, p. 655) are involved at some stage or other, for W. Jacobson[3]

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onfirmed by Ogorodny[3] and Fetischeva on ducks.

fowls there is a marked peak of mortality at the 6th day after hatching; could it be connected these adjustments?

should also be remembered that, as we have already seen (p. 594), there are shifts in the ciation curve of the chick embryo's haemoglobin analogous to those found in mammalian ises.

ctically nothing is known about the stomach in embryonic life, except that in mammals ic acidity is slight until some time after birth (Manville & Lloyd).

has shown that the argentaffin cells of the cardiac and pyloric portions of huma and pig stomach, which agree topographically with the distribution of th intrinsic factor, and which give a brilliant yellow fluorescence in ultra-violet light contain a pterine, probably xanthopterine or uropterine. That xanthopterin possesses some anti-anaemia activity in the rat has been stated (Tschesche & Wolf) and denied (Mazza & Penati[2]). Active preparations prepared from live (B. M. Jacobson & Subbarow; Subbarow & Jacobson; Mazza & Penati[1]) al contained pterine fractions among others. The preparations of Dakin's grou (Dakin & West; Dakin, Ungley & West), however, were of purely polypeptide nature, and there were apparently purines but not pterine in the active extract of the Swiss school (Karrer, Frei & Fritzsche; Karrer, Frei & Ringier). It doe not follow, however, that the active principle itself must contain pterine or purine In view of what has been said above (p. 631) regarding the function of the purine-containing nucleotides, it would be reasonable to think that the work of the polymeric purines, the pterines, might lie only in the formation of the principle. That the principle is complex is suggested from the report of Eisler, Hammarsten & Theorell that two active fractions with different effects can be separated b electrodialysis. As for the extrinsic factor, whatever it is, it is very stable to hea and to treatment with acids (W. B. Castle), and it is present in the undeveloped hen's egg (Miller & Rhoads).

In the light of all these facts, it is clear that the pterine metabolism of developing embryos in full haematopoiesis urgently invites study.

If, as indicated above, the blood picture in pernicious anaemia resembles that in early foetal life, it might be expected that the dissociation curve of pernicious anaemia haemoglobin would resemble the foetal type in having greater affinity for oxygen (cf. p. 599). This is actually the case. Korjuev & Belousov showed that the dissociation curve of the whole blood resembles that of foetal blood, and later Belousov established that this is a property of the haemoglobins concerned.

Many works have, of course, been devoted to the histology and morphology of the embryonic origin of haemoglobin and the blood circulation. Unfortunately, they have up to the present time contributed little towards our understanding of the mechanism of the process. As examples may be mentioned the papers of John on fishes, Słonimski[1] on amphibia and Hughes[1,2] and Grodziński[4] on the chick, to which the reader is referred for further information along such lines. Słonimski's work[2] was interesting in another connection, namely, as establishing that the presumptive blood-forming area on the ventral surface is determined at an early stage in gastrulation (see pp. 301, 378 and Table 22).

This may be the place to mention those invertebrates which possess porphyrin pigments for respiratory purposes or otherwise (cf. the monograph of Baldwin and the review of Barcroft[3]). One of these, the gephyrean worm, *Urechis*, has haemoglobin in its corpuscles (not in the plasma) and this turns to haematin giving them a brownish black colour. When the eggs are formed, granules of haematin are deposited in them, according to Baumberger & Michaelis, but whether or not as a store of porphyrin to relieve the strain of synthesis on the embryo is as yet unknown. The embryological aspect of all these porphyrin-containing organisms deserves much further study.

Another way of approaching the problem of haemoglobin synthesis is to investigate the metabolism of iron and copper in embryonic life, and this has been done on a considerable scale. The latter metal is important because it is now an

tablished fact that copper is in some way connected with the mechanism of
emoglobin synthesis (Waddell, Elvehjem, Steenbock & Hart).

Estimations by many workers (e.g. Lesné, Zizine & Briskas; Imaizumi, etc.)
ow that the yolk of a hen's egg contains about 170 mgm. iron kilo dry wt. and
copper about 7 mgm. kilo dry wt. There is no iron in the white, but about
mgm. copper kilo dry wt. All of this is absorbed into the embryo by the end
incubation and most of it is stored in the liver. Thus Loeschke found 1·3 mgm.
lo wet wt. of copper in the whole egg at the beginning of incubation; at the
d the liver had 13·5 mgm. kilo wet wt. and the remainder of the body only
mgm. kilo wet wt. Now we possess certain careful studies, such as those of
cFarlane & Milne and Szeynman-Rosenberg, from which the rate of accumu-
ion of iron and copper in the growing embryonic body may be calculated.
oth the metals show a bradyauxesis. On the dry weight, for the whole body,
on gives $k = 0{\cdot}87$ and copper $k = 0{\cdot}85$ (cf. Table 30); for the liver alone the
adyauxesis is more marked; iron gives $k = 0{\cdot}62$ and copper $k = 0{\cdot}67$. This, of
urse, implies nothing about the physiological importance of the metals at
fferent stages, for in the case of entities such as these which cannot be formed
nthetically by the embryo, bradyauxesis may only mean that there is not
fficient originally present to allow of tachyauxesis, unless a very rapid absorption
ere to take place, followed by complete cessation of absorption.* And this
pears not to happen. On the other hand a bradyauxesis of copper in the
bryonic liver of the pig has been established by Wilkerson (or rather, it may
inferred from his data, as McFarlane & Milne point out), although in this
se, where the maternal organism might have preferential access to foods rich
copper, the limitation has no such obvious origin.

That both iron and copper accumulate in embryonic livers, however, is the
reed conclusion of many investigators (Loeschke; Lenti[2]; McFarlane & Milne;
eynman-Rosenberg, and Imaizumi on the chick; Mayeda & Yamanuchi on the
bbit; Gruzewska & Roussel[1,2]; Roussel & Deflandre[1,3,5] on the cow; Hahn &
rman; Sheldon & Ramage[1]; Ramage, Sheldon & Sheldon; Adler & Adler;
zescu; Iob & Swanson[2] on man; and Lintzel & Radeff on a variety of mam-
ls). In some cases the liver of the foetus may contain 20 times the amount
ron or copper ever present in the adult liver. Unfortunately as the data given
often insufficiently full to allow of the necessary comparisons, and as all the
imation methods used are not equally worthy of complete confidence, we are
this, as in so many other similar problems, not as far advanced as the
ume of the literature would at first sight suggest. Some points are, however,
ar enough. Thus McFarlane & Milne and Lenti[2] showed that the non-haematin
n in the chick embryo liver rises from 10 % of the total iron present in the
er at the 11th day to 66 % at the time of hatching. This gives a picture of
destruction of blood pigment and the formation of bile pigments. Another
le-light on the bradyauxesis of the metals is obtained by the work of
megai, who estimated the iron and copper content of the blood of the chick

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ll inorganic raw materials of the chick embryo are not, of course, in this position. It by no
ns exhausts its lime supply.

embryo from the 9th day of incubation till hatching. Both declined, but coppe more markedly than iron before hatching and both equally afterwards.

After hatching or birth, the total amount of iron and copper in the bod temporarily declines.

3·462. Lipochromes

Leaving now the red porphyrin pigments we come to the yellow carotenoids.[*] Instead of the flat box-like tetrapyrrol molecule, we have to think of the lon partly unsaturated chains ending either free or in ionone rings (see formulae o p. 249). Connected with this topic, too, is the position of vitamin A.

The yellow pigments of the yolk of the hen's egg, which are very readil affected by the feed of the laying hen (Albright & Thompson; Henderson & Wilcke; etc.), have been known for many years to be carotenoids, but Kühn Winterstein & Lederer and Kühn & Smakula definitively identified the mixtur (previously called egg-yolk lutein) as two-thirds xanthophyll, identical with tha in leaves, and one-third zeaxanthin. Normally egg-yolks contain practically n carotin. Brockmann & Völker and Titus, Fritz & Kauffmann were able to show that in order to get into the yolk a carotenoid pigment must apparently have tw or more –OH groups, since neither violaxanthin, carotin, nor lycopin will ente the yolk when fed, except in so far as the hen can, according to Virgin & Klussmann, convert some of the carotin into xanthophyll. Brockmann & Völker state that certain eggs, such as those of gulls and storks, may contain a little astacin (see p. 249 and below). When given physaliene (the palmitic derivative o zeaxanthin, which occurs in certain plants) the hen cannot break it down, but wil readily put zeaxanthin itself into the yolk (Kühn & Brockmann). Quantitativ estimations by Terenyi; Mattikov and v. Euler & Klussmann[1] indicate that one yolk contains about 1·5 mgm. of the mixed carotenoids, with as little as 0·7 γ of carotin Besides these Gillam & Heilbron identified small quantities of cryptoxanthin especially if the hen's diet had contained maize. Lizard eggs follow this sauropsi type, if we may generalise from the observations of Manunta[5] on the chameleon

That the embryo can develop quite normally and hatch after the almost complete suppression of carotenoid pigments from the egg is now a classica observation (cf. *CE*, p. 1379). Particularly interesting in this connection is the work of Wald & Zussman on the origin of the carotenoid pigments of the chick's retina. By the time of hatching the retina contains three kinds of droplets; red ones containing astacin, golden-yellow ones containing xanthophyll, and greenish-yellow ones containing a hitherto unidentified hydrocarbon. All these appear during the last week of incubation, and the first and third types must certainly involve synthesis on the part of the embryo.

Astacin has already been mentioned in some detail in other connections (see pp. 210, 250). Green when combined with protein, it is pink when free.† Dis-red in lobster eggs by Kühn & Lederer, it was found by Fabre & Lederer and n & Salomon in the eggs of a wide variety of crustacea, and by Kühn, Lederer eutsch in those of the spider-crab, *Maia squinado*, where it is accompanied, by xanthophyll, but by β-carotin. The same combination occurs in teleostean eggs such as that of the cod, according to Emmerie, van Eekelen & Wolff, v. Euler, Gard & Hellström. That this cannot hold good for all teleosts, ever, would seem to follow from the work of Manunta[7], who studied the eggs wo races of goldfish, *Carassius*, a red and a yellow. Both kinds of egg had nly xanthophyll esters, but the red one had astacin as well. The eggs of hnid crustacea would bear further investigation in this connection; all we w from a note of Teissier[3] is that some races have red eggs (due to the presence aemoglobin or erythrocruorin?) and others have green ones, due to a caro- id pigment the nature of which is unknown, but which is only formed when tin is present in the diet. Finally, the eggs of the silkworm moth have been subject of a good deal of Italian work. First described by T. Teodoro; Duce, Tirelli[4], the work of Manunta[1] has shown that all races have xanthophyll and e have carotin as well. But the most interesting fact, and one which pre- ably accounts for the wide variations in egg-colour which silkworm races bit, is the presence of the plant pigments, flavones, in the eggs of certain s discovered by Jucci; Jucci & Manunta, and Manunta[1,3,6]. The bright green nent, formerly called bombichlorine, thus turned out to be a flavone. h an explanation may also account for Gerould's bright blue caterpillars, a ant race of the normally green pierid *Colias philodice*.

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[*] See the monograph of Zechmeister.

† The name "ovoverdin" is proposed for the green combination. The pigment described b Yamamoto (p. 210) in the eggs of the worm *Ceratocephale* is probably a similar lipochrome-protein; its colour changes reversibly from lemon-yellow to green according to the conditions o illumination.

ery little is known of the carotenoid metabolism of the mammalian embryo.* thophyll has been identified by v. Euler & Klussmann[2] in the human placenta, Clausen & McCoord have noted that the carotin content of human foetal d seems to be exceptionally low. Kühn & Brockmann found that at the inning of gestation there are equal amounts of xanthophyll and carotin in lacenta; towards the end there is much more of the former than the latter. his remains at present quite enigmatic.

e metabolism of vitamin A (formula on p. 249) in eggs seems to be rather pendent of the other polyene compounds. Brockmann & Völker found that mount of it in the yolk was not much varied by the revolutions which they ted in the pigments of the diet. On the other hand, there is no doubt that nature of the diet of the laying hen can profoundly affect the vitamin A ent of the egg (Russell & Taylor; König, Kramer & Payne; Bearse & Miller; ckshank & Moore). At the beginning of development an average yolk will ain some ninety colour units of the vitamin, and as development proceeds is all absorbed by the embryo, though very slowly, so that the greater part e embryo's supply is still to be found in the spare yolk at the time of hatching lmes, Tripp & Campbell). As we have already seen (p. 23 and pp. 227 ff.), oper supply of vitamin A is essential for normal morphogenesis, development hatchability. This explains the great loss (45 % of the initial store) of the min during the development of the chick, noted by Suomalainen[1] as well as by the American workers. Aykroyd & Sankaran, testing the growth-promoti power of plasma from vitamin-A-deficient rabbits on chick embryo explan found it to be noticeably low, but it is extremely doubtful whether the wh effect of vitamin A on development is explainable in terms of growth.

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* The monograph of Lütgerath has not been accessible to me.

As regards mammals, something has already been said (p. 77) about transmissi of vitamin A from maternal organism to foetus or new-born. Placentas contain (Gaehtgens[2]; Dann[2]). The reserves of the vitamin in the liver are liable to be lo at birth (Busson & Simonnet on dogs; L. K. Wolff[2] on man). Mason[2] has ma an elaborate study of the interferences with gestation in the rat which su normality in vitamin A entails; they differ from those characteristic of vitamin deficiency (Evans, Burr & Althausen; Urner) in various ways, partly because th uterine wall in A deficiency generally becomes infected during the resorpti changes.

One very interesting problem concerning vitamin A is its synthesis by teleostea fishes. Just as for the chick vitamin C is a synthesisable hormone rather than vitamin, so it appears that for the cod embryo vitamin A is a synthesisab hormone. McWalter & Drummond found, in conformity with what has bee said above, that the eggs of the trout contain a carotenoid very similar if no identical with carotin, and that this decreases continuously until the end of th yolk-sac stage, while the vitamin A content continuously increases. Such a powe of synthesis is needed to explain the appearance of vitamin A (as in cod liver oil at the fish stage in the marine food-chain (cf. Elton).

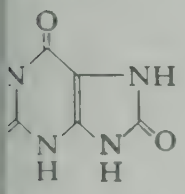
3·463. Flavines

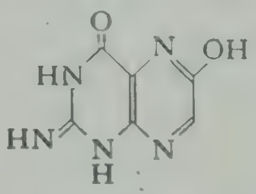
The next group of pigments to be discussed is that of the greenish yello fluorescent water-soluble flavines. As has already been noticed (p. 208) they ar found free as well as in various combinations with phosphoric acid and protei which have co-enzyme and vitamin properties (R. Kühn[1]). Ovoflavin was isolate from egg-white of the hen's egg by Kühn, György & Wagner-Jauregg almos simultaneously with the discovery of lactoflavin in milk by Ellinger & Koschara They are now considered to be the same substance: 6 : 7-dimethyl-9-(*d*-ribityl) benz-iso-alloxazine (Kühn & Wagner-Jauregg; Karrer & Schöpp; see formula o p. 209). Without the ribitol, the alloxazine is a reversibly reducible substanc (K. G. Stern[1]); plus ribitol, the compound (lacto- or ovo-flavin) is identical wit vitamin B_2 (hence the significance of the presence of this vitamin in the egg white); vitamin B_2 plus phosphate is the co-enzyme, and hence (on the Warbur view) the prosthetic group, of yeast flavoprotein, i.e. an important dehydrogenase (R. Kühn[2]). Like other flavines, such as urochrome (Stern & Greville ovoflavin catalyses cell respiration (Adler & v. Euler). Nothing further is know about its function or metabolism in the chick embryo, but Neuweiler[3] find 5γ/gm. in the human foetal liver (of which 70 % is bound) as against 2γ/gm in the placenta (of which only 40 % is bound). Much remains to be done o this subject. Apart from this we have only a few fragmentary observations o the presence of flavines in the embryos of selachian fishes (Fontaine & Gurevitch) and lepidoptera (Drilhon & Busnel) to which as yet it is impossible t attach meaning.

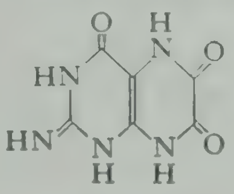
·464. Pterines

o far as we know at present, the pterine pigments are confined to insects, ough small amounts probably occur in all animals, where quite possibly they e some important function not yet clear.* In 1889 F. G. Hopkins[3] made the sical discovery that pigments which are derivatives of purines occur in the gs of butterflies. These pigments account for much of the cream and yellow oration of insects, such as that of the wings of the cabbage-white butterfly, *is*, or the yellow bands of the wasp, *Vespa*; and they must have some relation the well-known uricotelic and guanotelic metabolism of insects and arachnids p. 50 above). The cream pigment was at first thought to be uric acid itself, but r the school of Wieland (cf. Wieland, Metzger, Schöpf & Bülow) considered pterines to be tripurine polymers or condensation products. The most recent v, however, is that xanthopterine and leucopterine differ from purines only in t the pyrimidine ring is condensed with a pyrazine instead of an iminazol ring eland & Purrmann; Purrmann). It will be noted that they form thus an interdiate group between the purines and the alloxazine ring of the flavines (see p. 209).

ll that we know about the ontogenetic aspect of the pterines is derived from work of Becker[3], who studied the formation of xanthopterine in the developing wasp, and found that it arises much later than the melanin. But there is an interesting parallel in this field to the action of inductors across genetic boundaries p. 341). In 1895 Hopkins[4] showed that when an insect mimics the colour nother insect, it does so by using the pigments characteristic of its own group, pterines instead of carotenoids. Here the stimulus is that of natural selection, if the organism reacts, it can only do so in terms of its own enzyme proteins. years later, Sir F. G. Hopkins[5] continues still these studies.


Uric acid


Xanthopterine


Leucopterine

·465. Melanin

word may finally be said about melanin, though the reader must be referred ie genetical section (pp. 405 ff.), where melanin formation was discussed in her connection. The subject, after much work, is still in a rather unsatisory state, since apart from histological and morphological studies which, e, are incapable of advancing it much (e.g. Thumann on fishes; Dorris[2] on chick; du Shane on amphibia; Peck on the rabbit; Makarov on the chick ryo retina), we know practically nothing of the nature or origin of the strate of tyrosinase in embryos, nor what mechanisms control the formation deposition of melanin. According to Dawes, the amount of melanin in the egg increases considerably between blastula and neurula stages.†

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or example, a connection with the anti-anaemia factor in the stomach of primates is suspected, e have just seen (p. 650).

this connection the experiments of M. R. Lewis[2] and Figge[3] are interesting. They found certain indophenol dyes, such as phenol-indophenol or *o*-cresol-indophenol, suppressed the al development of melanin in pigmented amphibia such as the axolotl. Figge proved that effect is not an indirect one by way of the pituitary, and later that the dyes exert an itory effect on tyrosinase itself (Figge[4]). In the course of these experiments it appeared melanin itself is capable of being reversibly reduced to a light brown or tan colour (Figge[5,6]).

The darkening and hardening of the insect cuticle at various developme
stages has been the subject of much recent work. The protein has been stud
both chemically (Trim) and physically (Fraenkel & Rudall). Pryor believes t
a veritable tanning process takes place, a dihydroxyphenol being secre
together with the protein, and then denaturing it by forming aromatic cr
linkages. The tanning process would be closely connected with the mela
formation since the parent substance for both would be the same; dihydro
phenylalanine brought in the blood stream.

3·5. Polarity

In concluding this book, it is essential to say something about the biochemi
approach to the fundamental and difficult question of polarity, perhaps the cent
puzzle of embryonic development. How do the axes of symmetry originate, a
how does morphogenesis conform to them? Although I shall avoid going o
the ground already covered in a former book (*Order and Life*, ch. 3), a cert
amount of repetition will hardly be avoidable. The argument there put forwa
was as follows.

A logical analysis of the concept of organism leads us to look for organisi
relations at all the levels, higher and lower, coarse and fine, of the living structure
Biochemistry and morphology should, then, blend into each other instead
existing, as they tend to do, on each side of an enigmatic barrier. The chemic
structure of molecules, the colloidal conditions in the cell, and the morphologic
patterns so arising, are inextricably connected. It is easy to find instances
the way in which organisation may appear already at the chemical level.
are driven to the view that the living cell possesses as complex a set of interfac
oriented catalysts, molecular chains, reaction vessels, etc. as the organs, tissues a
other anatomical structures of the whole organism.

3·51. The organisation of cell protoplasm

Indications of the organised nature of the cell protoplasm are so numero
and well known that only a few may be mentioned. Thus it is significant t
Vlès & Gex, examining the transparent sea-urchin egg in the ultra-violet spectr
meter, obtained an absorption curve which was not typical of protein, althou
such substances must undoubtedly form by far the largest proportion of the so
matter present in the eggs. Only upon cytolysis and the death of the cell did t
characteristic protein curve appear — an indication that some curious structu
state is present in the egg. Then much of value has come from the vigor

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* An amusing essay, in the style of Veblen or Mencken, might be written on the influenc
sectional interests on the development of scientific terminology. Morphologists have long ten
to speak of the "higher" (morphological) as opposed to the "lower" (chemical) levels of organ
tion, but the balance may be redressed by speaking of "coarser" and "finer" levels. So
physical chemists, wishing to avoid the undertones of the word "superficial", speak of "in
facial" reactions, as opposed to those going on in the "bulk phase", thus ingeniously giving
impression that the classical methods of chemistry are really only fit for peasants. "Bulk analy
is a term also used by morphologists who wish to suggest that most of biochemistry consist
analysing dead materials on the "raw fibre" and "humin nitrogen" level. It is regrettable
this temptation seduces even the elect (e.g. Weiss[16], *Principles of Development*, p. 187). And
to be hoped that the present book will have helped to demonstrate the absurdity of such an outl

of researches which followed the perfection of the micro-manipulator,
ly due to the work of Robert Chambers, as an efficient tool for handling
ted single cells. When Hiller[2] made experiments on amoebae with the
tics, ethyl alcohol, chloretone, chloroform and ether, he found that,
ugh their action was marked on immersion, they had much less action
n micro-injected into the cytoplasm. In all concentrations chloretone
ased the fluidity and streaming movements of the interior. Alcohol
t cause a reversible coagulation. Still more remarkable was the result of
ck, who micro-injected solutions of picric acid, and found that concentrations
ns which coagulated proteins in the test-tube had no such action in the
ior of the living *Amoeba*. Only if there was a local injury at the point of
tion did the picric acid coagulate the protoplasm. In contrast to the non-
ity of the reagent within was its extreme toxicity when applied to the
nal surface. An analogous observation is that if an oil-drop be micro-
ted into a cell, it can be swollen and shrunk by changing the pressure at will
ng as the cell lives, but immediately upon cytolysis a film of adsorbed protein
s on the droplet causing a wrinkling if oil is withdrawn into the pipette,
mbers & Kopać[2]).

oreover, the importance of intra-cellular organisation in explaining certain
bolic and respiratory phenomena cannot be overlooked. It has been well
hasised in a review by Korr[3], who mentions most of the classical cases.
rganisation of the cell structure in cytolysis destroys some former enzyme-
trate contiguities necessary for the life of the organism, and sets up some
ones, usually not geared to the maintenance of life, nor capable of being so.
s in sea-urchin eggs cytolysis almost entirely destroys respiration. In lysed
eria the haematin catalysts remain active but the dehydrogenases are
oyed, hence the respiration also falls nearly to zero. Conversely, where
iration is normally restrained, interference with cell structure may much
se it, as in the bruised portion of a mushroom, or the amphibian gastrula
tolysis (p. 589). In this connection we shall recall the "damped" meta-
n of the unfertilised echinoderm egg, the grasshopper embryo in diapause,
he ventral ectoderm of the amphibian gastrula (p. 198). To these examples
llowing may be added. The echinochrome granules of the sea-urchin egg
no fading when the egg is subjected to anaerobiosis, although the dye is
n to be reversibly reducible, and the intra-cellular reduction potential is
n to change. The hermidin of dog-mercury (*Mercurialis perennis*) exists in
lant in a state of 95 % reduction, although bubbles of oxygen are actually
formed photosynthetically in the same cells. Luminous animals at death
out a continual steady luminescence, the "death glow", indicating that the
erin-luciferase contiguity can no longer be controlled. Many plants darken
ackness on death, e.g. the false indigo (*Baptisia tinctoria*), but attempts to
uce this on the living leaf by forcing oxygen into it under high pressure
lb. sq. in.) fail because, until the cell structure breaks down, chromogen
xidase are held firmly apart. Lastly may be mentioned the numerous cases
hich important enzymes, such as cytochrome, desmokathepsin, or the

glucolytic enzyme system, are so closely associated with cell structure. Th suggests a micro-morphological arrangement of them within the cell.

Against the conception of high organisation in protoplasm might arise, hov ever, the fear of a revival of completely discredited theories of the "bioge molecule" type, were it not for the fact that we now possess much more powerf means of rendering living wholes "transparent". Among these the ultra-viol spectrometer, which has achieved such success in the location of nucleic aci within the cell (p. 636), may be mentioned, and especially the X-ray analysis polysaccharides (Sponsler) and of proteins (Astbury; Astbury & Bell; Astbu & Street; Astbury & Woods). The study of highly anisometric molecules b other methods will also be of great importance for embryology; in this respe the reviews of Staudinger should be consulted.

3·52. Fibre-molecules and "dynamic structure"

The importance of the work on the crystal structure of animal fibres can hard be overestimated. Is not biology as a whole very largely the exploration of fib properties? It is now certain, for instance, that in the case of muscle the con tractile mechanism is essentially a molecular contraction, and with this there enc a state of ignorance not much less intense than that which existed in the time Descartes and Borelli. But we are especially interested in the egg-cell. It ma be a far cry from the macroscopic fibres hitherto mostly studied to the intra cellular lattice of protein molecules forming the cell architecture, but there is n reason to suppose that the one is less open to investigation than the other. An the subject has real importance in connection with the polarity and symmetry the fertilised egg.

Embryologists have long been impressed with the capacity of eggs to develo normally after their contents have been thoroughly stratified by centrifugal forc The centrifuged amphibian egg, for instance, will show a mass of yolk-granul at the centrifugal pole, a cap of fat at the opposite one, and an intermedia transparent protoplasmic layer. Conklin[2], in particular, has drawn attention the possible existence of a "spongioplasmic framework" not destroyed by centr fugation, in spite of the egg's "ballast" being movable through its meshes. There is much evidence, indeed, that the egg contains a "framework of visci protoplasm which is so elastic or contractile that it recovers its normal form aft distortion". On the other hand, egg cytoplasm can be remarkably fluid, showin churning movements as cinematographed by J. E. Harris; and Chambers' descrip tions[4] of the blastomeres during cleavage show convincingly that the cytoplasm may for a time at any rate flow in currents like a liquid.† The protein chains the cell's web or lattice must therefore be pictured rather as connected at man points by residual valencies and relatively loose attachments, *so that they ca as it were, snap back after disarrangement*, whether the disarrangement is normall periodic or experimentally induced. We may call this "dynamic structure". is probably connected with those isothermal reversible sol-gel transformatio which go under the name of thixotropy (described in Freundlich's monograph ugh doubtless much more complex than any of the "inorganic" examples taining few components. A thixotropic gel liquefies if shaken or stirred, r returning to its previous consistency. Angerer has shown quantitatively t amoeboid protoplasm does just this.

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* There is of course also the special structure of the egg's surface, reviewed by Harvey & Daniel

† Or perhaps like a liquid crystal?

ike Pascal's being suspended between two infinites, protoplasm with its arities hovers between the perfectly liquid and the perfectly solid. At the er extreme from the liquid cytoplasm just mentioned are the eggs of the ne-shrimp, *Artemia*, which can be held in high vacuum over phosphorus ntoxide for a year (Needham & Pirie) or cooled to the temperature of liquid air held at 10^{-6} mm. Hg for six months (Whitaker[20]), and yet subsequently give mal development. We are reminded of the crystallisation of some of the plant us nucleoproteins. In certain cases, therefore, the "dynamic structure" can k into highly dehydrated form without permanent injury.

The thixotropy of protoplasm is strikingly illustrated by the remarkable and l-known observation of Kühne, who in 1863 saw a small nematode worm ving actually within an amphibian muscle fibre. "The movements of the rm", writes Freundlich, "caused characteristic changes in the aspect of the ations; if the animal moved parallel to the fibre axis, the striations moved the hairs of a brush, touched slightly by a solid body; when the nematode pierced a striation, the latter closed up again, so to say, behind the worm's . The ease of movement seems to indicate that the mass of the interior is d; the well-defined, reversibly rearranged orientation of the striations looks re like the behaviour of a solid body. Both facts are explained by assuming hixotropic state." Other later observers have reported similar facts; thus agi found an actively moving bacterial parasite within the protoplasm of the tozoon *Spirostomum*, which seemed to have no deleterious influence on its t. Protoplasmic thixotropy has also been studied by Fauré-Fremiet[1] on the oebocytes of marine annelids, which have well-marked active and quiescent s. He added the significant finding that only in the "sol" state does the plasm show the nitroprusside reaction for free sulphydryl groups, not in the " state. Reversible denaturation of the proteins may therefore be involved p. 423).

lsewhere Conklin[5] describes how the nuclei, centrospheres, yolk inclusions, of invertebrates' eggs usually come back slowly to their normal places after action of pressure or centrifugal force has ceased. Wintrebert[8], too, speaks of need for the hypothesis of a "cyto-squelette" or "trame spongioplasmique". 324 shows the familiar failure of centrifuging to affect the micromere pole chinoderm eggs. Schleip, again, at the conclusion of his encyclopaedic work embryonic determination, says: "In every attempt at the explanation of rity and symmetry in the egg, some as yet unknown property of the proto- m has to be introduced. To avoid giving it any new name, which could only rbitrary and tentative, I will call it 'intimate structure' (*Intimstruktur*)." Or n: "An intimate structure is present, i.e. a morphologically invisible, specific erty of the cytoplasm, the manner of working of which we do not yet erstand. To this must the difference between spiral and radial cleavage be referred, this determines too the direction of bilateral-symmetrical cleavage, an governs the planes of subsequent mitoses." Przibram[5], in his work on the cryst analogy, is still bolder, and does not hesitate to hypostatise the Thompsonia co-ordinates (p. 531). "Such systematic deformations", he says, "may mos easily be explained on the assumption of an organic space-lattice. Just as by th substitution of radicals in eutopic series of crystals, deformations may be obtaine because the effect is the same on all parts of the lattice, so the substitution of one protein combination for another would bring about a parallel change in the organic space-lattice."

This is not nearly so extravagant as it sounds, if we remember the elastic properties of protoplasm. An entanglement or "brush heap" of interlacing crystalline fibres or amino-acid chains would be elastic; an emulsion of spherical particles would not (Seifriz[3]).

The "cell skeleton" is, however, by no means easy to study. Among interesting recent attempts to do so are those of Howard[1] and of Vlès. Howard studied the apparent viscosity of sea-urchin egg protoplasm at various rates of shear by observing the rate of movement of granules under centrifugal force. No unequivocal plasticity was shown by the egg at rates of shear so low that the granules were moving at velocities comparable to their own migration velocities (i.e. their velocities of return to place after centrifuging). No continuous structure, it was concluded, was present which could significantly affect diffusion, nor, *a fortiori*, be the basis of polarity. But it is uncertain, as Pfeiffer[1] has pointed out, whether what we are looking for could be studied by an centrifuge method. Vlès, again, investigated the moduli of elasticity and rigidit in the sea-urchin egg as it becomes a sphere after deformation to a sausage-lik shape. His results do not exclude the possibility of the structure we are envisagin One thing at least is clear, namely that proteins within the protoplasmic structu do not behave like isolated proteins in Svedberg sedimentations. The classic observations of Beams & King[1] showed that *Ascaris* eggs may be subjected t centrifugal forces of 400,000 × *g* for a short time, and 150,000 × *g* for four day continuously, and yet later respire and develop normally. Gortner[3] has acutel remarked that if we had no microscopes with which such eggs had been ex amined, they would have been described as uniform-sized protein micelles c enormous particle-weight, and classed among the globular proteins. Baitse replied that this is no criticism of either protein chemistry or embryology, an that we are indeed forced to envisage the egg-cell as a single extremely elabora

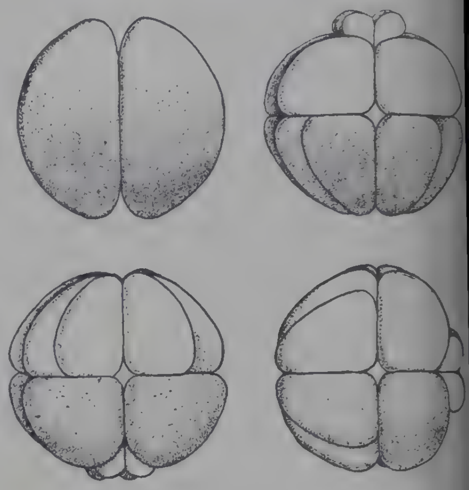

Fig. 324. Persistence of the primar axis in the echinoderm egg (*Arbacia* in spite of the rearrangement o visible substances in the cytoplasm After centrifuging, the egg become stratified with fat at the centripeta pole, then clear cytoplasm, then yolk granules with increasing amounts o pigment. The first cleavage (top left is always at right angles to the strati fication, but the micromeres ar always formed at the vegetal en of the original axis, whether thi coincides with the centripetal pol of the centrifuged egg (top right its centrifugal pole (bottom left), o its side (bottom right). (Fron Morgan, redrawn by Huxley & d Beer.) Cf. Fig. 267.

ein complex, holding within itself many other types of molecule, as even the talline virus "molecules" or micelles almost certainly do.[*]

'he extraordinary tendency of protozoa to swim in spirals, some right and e left, leads Schaeffer to postulate a stereochemical difference in the protein iework, and the sinistrality and dextrality of molluscan eggs in their vage, which corresponds with their shell rotation, may be referable to eochemical differences in their proteins, though this has not been proved vcott, Garstang & Diver). Rand & Hsu have a passage of particular interest. cribing the motion of amoeboid cells, they say: "In watching the nucleus is perplexed by the fact that it is so freely movable within limits which are io way visibly defined. Apparently freely immersed in a labile and actively ing protoplasm, why is its position not completely at the mercy of the ents? It must possess some highly elastic anchorage. It is as if a slightly yant sphere, immersed in a strongly flowing stream, were anchored by tively slender and extremely elastic cables."

rotoplasm undoubtedly shows anomalous viscosity. Pfeiffer[3] and others have id that the velocity of its forced shearing flow, as in a capillary tube, increases ı increasing force at first slowly, later more quickly. For Newtonian liquids relation, however, is linear. Also protoplasm has a "yield value", i.e. requires ecially large initial force to start the flow (cf. thixotropy). The viscosity of liquids is unaffected by pressure, but that of protoplasm greatly decreases he pressure increases. The anomalous flow of protoplasm is paralleled by iy non-living colloids, as Lawrence's paper[1] shows, if they consist of aniso- ric particles which mechanically interfere with each other's motion.

·53. The paracrystalline state

'he aspect of molecular pattern which seems to have been most under- mated in the consideration of biological phenomena is that found in liquid als (cf. the books of Rinne and W. J. Schmidt[1,5]). It is likely that pro- ; in the discovery of the "leptonic fibres"[†] of which we have been king will be delayed until more knowledge is available of the peculiar form der which liquid crystals exhibit. Liquid crystals, it is to be noted, are not ortant for biology and embryology because they manifest certain properties h can be regarded as analogous to those which living systems manifest dels), but because living systems actually *are* liquid crystals, or, it would be e correct to say, the paracrystalline state undoubtedly exists in living cells. doubly refracting portions of the striated muscle fibre are, of course, the sical instance of this arrangement, but there are many other equally striking ances, such as cephalopod spermatozoa (W. J. Schmidt[2]) or the axons of ve cells (Schmitt; Schmitt & Bear) or cilia (McKinnon & Vlès) or birefringent ses in molluscan eggs (Pfeiffer[2]) or in nucleus and cytoplasm of echinoderm eggs

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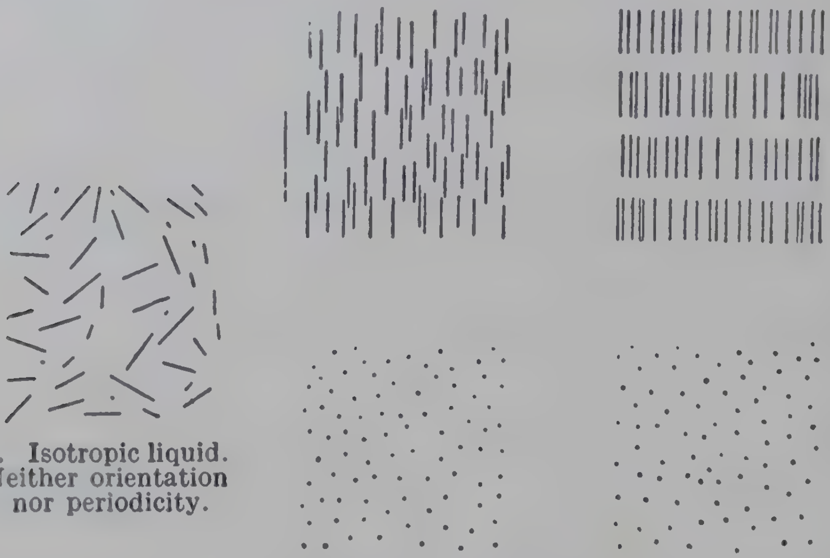
. Pirie's thoughtful discussion of this point.

iis expression is taken from the suggestion of Rinne (Faraday Society Symposium, *Liquid tals*, p. 1029) that for international purposes parallel words are needed for the German *ibaulehre*", "*Feinbau*". He suggests "leptology", "leptonic", from λεπτος, delicate, fine.

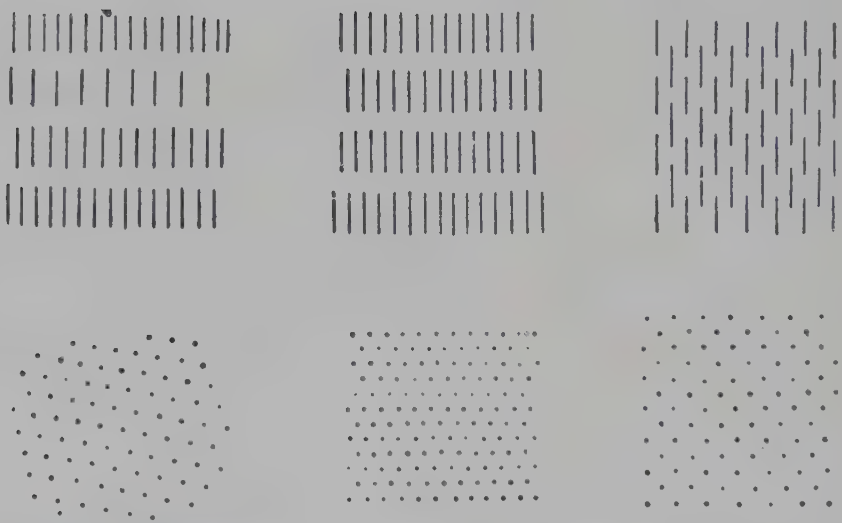
(W. J. Schmidt[4]). Even in the amphibian egg the yolk-platelets are anisotr (W. J. Schmidt[2]). And it is very probable that the paracrystalline state exis many phases in the cell whose scarcity or position has so far rendered t



I. Isotropic liquid. Neither orientation nor periodicity.

II. Nematic. Orientation without periodicity. Above, viewed perpendicular to molecular axis; below, parallel to molecular axis.

III. Normal Smectic. Orientation and molecules in equispaced planes with no internal periodicity. Above, viewed perpendicular to molecular axis; below, parallel to molecular axis.



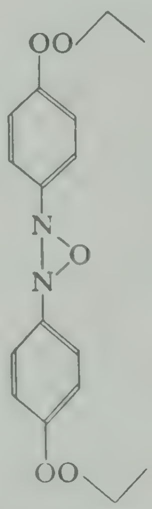
IV. Low Temperature Smectic. Each layer with two dimensional periodicity but unrelated to other layers.

V. Crystalline.
(*a*) Smectogenic. (*b*) Nematogenic.
Complete orientation and three dimensional periodicity.

Fig. 325. Types of liquid crystals.

immune from investigation and invisible when the living cell is placed in po ised light. As Rinne points out, the Langmuirian oriented film of fatty acid water may be regarded as paracrystalline, and this is certainly a close relat of the oriented films within the cell or the egg.

cording to our ordinary conceptions, when a solid is heated it melts on ing a certain temperature, passing immediately into the state of an isotropic d, where the molecules are arranged at random according to kinetic theory. , however, is much too simple for many substances, which on the contrary through a succession of “mesoforms”, conditions where a certain amount of d-like flow is possible, and yet where there is within the liquid a definite regular arrangement of molecules. These mesoforms are now classified as ws. Remembering that all paracrystalline substances have elongated mole- ; several different cases may arise (see Fig. 325). In I the molecules are letely at random; this is the true isotropic liquid state. In II all the molecular are parallel, but the centres of the molecules are as irregularly arranged and ee to move as in the first case. This is called the *nematic* state. In III also nolecular axes are parallel, but the centres of the molecules have lost one ee of freedom and are now restricted to a set of regularly spaced parallel ices. This is the *smectic* state. In IV the smectic state is still further ordered aving the molecules regularly arranged within each layer. V gives the con- ns in the crystalline solid state. The molecules are parallel and their centres a regular three-dimensional network. A smectogenic crystal has its molecules ged in planes (V *a*); a nematogenic crystal has them arranged so as to inter- each other (V *b*). In addition to these states there may be others, and it is esting that one has long been recognised, the most complicated of all, called *steric*, since it is typically shown by the fatty acid esters of cholesterol. The d crystals of plant viruses seem to be intermediate een type II and type III (Bernal & Fankuchen), since nolecules are packed with hexagonal two-dimensional larity at right angles to their length, but there is still vidence of regularity of molecular arrangement in the tion of their length. With pure substances, such as -*p*-azoxybenzoate, paracrystalline phenomena are most to be observed in melting, but with two or more onents in a system, the temperature factor may be ced by changes in the concentration of the solvent or rsing medium. Most of the protein, fat and myelinic ance of the cell probably exists in these states, but this y directly visible when all the molecules are oriented ormous swarms in one direction, as in muscle fibrils. paracrystalline state seems the most suited to bio- al functions, as it combines the fluidity and diffusibility of liquids while erving the possibilities of internal structure characteristic of crystalline s (Lawrence[2]).



Ethyl-*p*-azoxybenzoate

his has been very well put by Bernal. “The biologically important liquid als”, he said, “are plainly systems of two or more components. At least one be a substance tending to paracrystallinity and another will in general be r. This variable permeability of liquid crystals enables them to be as effective emical reactions as true liquids or gels, as against the relative impenetrability of solid crystals. On the other hand, liquid crystals possess *internal structur* lacking in liquids, and *directional properties* not found in gels. These two properties have far-reaching consequences. In the first place, a liquid crystal in cell, through its own structure, becomes a *proto-organ* for mechanical or electrica activity, and when associated in specialised cells (with others) in higher animal gives rise to true organs, such as muscle and nerve. Secondly, and perhaps mor fundamentally, the oriented molecules in liquid crystals furnish an ideal mediu for catalytic action, particularly of the complex type needed to account for growt and reproduction. Lastly, a liquid crystal has the possibility of its own structure singular lines, rods and cones, etc. Such structures belong to the liquid crysta as a unit and not to its molecules, which may be replaced by others withou destroying them, and they persist in spite of the complete fluidity of the substance They are just the properties to be required for a degree of organisation betwee that of the continuous substance, liquid or crystalline solid, and even the simples living cell."*

It has been known for a long time that many constituents of the living ce manifest remarkable interfacial properties when in contact with water (lipins an sterols); this knowledge long antedates our information on the crystalline characte of protein chains. The "myelin figures" produced by wetted lecithin are classical They are undoubtedly connected with the solubility of the phosphoric acid an glycerol groups in the molecule and the insolubility of the hydrocarbon chains of the fatty acids. First, spheres with optically positive radii are formed, and from these there grow out excrescences and long sluggishly writhing masses, often wit remarkable turns, twists, spiral wrappings and swellings. As the water-conten is increased the double refraction falls to a low value. Leathes, who pleads fo the view that protein structure alone will not be adequate for the understandin of intra-cellular structure, has cinematographed these myelin forms under man different conditions.

3·54. Rigidity in paracrystalline mesoforms and in embryonic structures undergoing determination of spatial axes

It has been pointed out by Lawrence & Rawlins that the mesoforms can b defined in terms of dimensions. A solid is rigid in three dimensions, a smecti mesoform in two, a nematic mesoform in one, an isotropic liquid in none. Thi can easily be appreciated by reference to Fig. 325. On heating, melting occurs i the three dimensions in turn; the three different melting-points being the temperatures at which the three vectorial forces of adhesion are overcome by therma agitation. This formulation, though not quite accurate, draws attention to th fact that the basic observation on mesoforms is that several melting-points succeed one another in the transition from solid to liquid. Now there is a similarity here between these successive stages of dimensional rigidity and the very curious phenomena seen in the determination of limb-buds in amphibia.

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* Cf. Staudinger's "Darüber hinaus aber weisen makromolekulare Stoffe eine Fülle von *nur bei ihnen* möglichen Reaktionen auf. Darauf fasst ihre Bedeutung für den Biologen."

n order to explain this we shall have to take up again the story of limb-bud
erentiation at the point at which it was left on p. 308. From the fundamental
k of R. G. Harrison[5] in 1921 we know that when discs of the outer wall of the
y, representing the buds of the future limbs, are transplanted at a certain stage,
found that the original anterior edge of the bud always produces the pre-axial
t of the limb. So a limb-bud of the left side, planted the right way up on the
it side of the embryo, will develop into a limb with the elbow pointing forwards
ead of backwards, if it is a forelimb. On the other hand, if the disc is rotated
hat the original dorsal edge of the bud is ventral, the original anterior edge
be anterior again, and a normal limb develops. Therefore the dorso-ventral
s can be inverted without producing rearrangement, but not so the antero-
terior axis. The medio-lateral axis can also be inverted at this stage with
unity, i.e. it does not matter whether the limb-bud is attached to its new
ation proximally or distally. This means that the dorso-ventral and medio-
ral axes are still plastic, while the antero-posterior axis is determined. Later
they also become determined. Here, then, we have successive stages of
nensional determination. Just as the liquid crystal passes through stages of
dity in one, two and three dimensions, so the limb-bud passes through stages
determination in one, two and three dimensions. The analogy here may, of
rse, be superficial, but it is sufficiently striking to warrant attention.

o the above account some further facts may be added. Harrison's work was
tinued by one of the investigators of his school. In Swett's[2] first paper* the
ual times at which the successive determinations take place are given us:

Dimension of space	Time of determination Harrison stage
ntero-posterior axis	Before 20 (probably from the first appearance of the limb district)
orso-ventral axis	Newt 29; axolotl 33–34
edio-lateral axis	36–37

e stages vary somewhat according to the species. The determination of the
o-ventral axis cannot be delayed by transplanting the limb-bud to a younger
(Swett[4]), nor can it be accelerated by transplantation to an older host (Swett[6]).
arising out of an earlier experiment of Nicholas, it was found that the deter-
ation of dimensional polarity does to some extent depend upon the site to
ch the limb-bud is transplanted. If placed in non-harmonic orientation on
flank at stages prior to the determination shown above, the limb-bud will
ave *ortsgemäss* as it should, and hence will plastically assume harmonic
tions; but if placed more dorsally over the somites, it will behave *herkunfts-*
äss at an unexpectedly early stage (Swett[7]). This seems to be due to a failure
he re-orienting influences, which otherwise would operate, to reach the back
n the flank. It afforded Swett[8] an opportunity of finding out how long it takes
the prospective polarity of a limb-bud to be changed in the direction of
monic relations after transplantation on to the flank. Limb-buds were trans-
nted to the flank and then removed after varying intervals of time to the back.
e result showed that the determination requires very little time, being com-
ed almost as soon as the limb-bud has healed into its first new position.

* Swett[5] has also contributed a valuable review of this subject.

The successive determination of the spatial axes seen in the limb buds al holds good of the ear-vesicle; see Fig. 326 (Harrison[8,15]; E. K. Hall[1]). At its fir appearance, the axolotl ear-vesicle is "isotropic", a state which continues up t stage 19, when determination of the antero-posterior axis sets in. At stage 25 th dorso-ventral axis becomes determined, and finally, probably at stage 29 or late the medio-lateral axis is determined.

Several points of some importance arise out of this. In the first place, it i rather remarkable that the axes, or at any rate the first two, are determined befor the ear-vesicle ceases to be an equipotential system. As late as stage 24 it i possible to fuse two ear-vesicles into a single complete organ. Secondly, it i remarkable that the limb-bud and the ear should both conform to the sam succession of axis determination, in view of the radical difference between the as embryonic systems. "The limb", writes Harrison[15], "is a mesenchymati rudiment, out of which muscles and skeleton are segregated; the ear is an epitheli system, consisting at first of a circular plate of cells, or placode, from which th mature organ develops by invagination and folding. While these changes can b accomplished only by a complicated shifting of cells, roughly speaking, a circul plate of cells becomes transformed into a sphere." Since the tail also conform to the same succession of axis determinations (Morikawa), we are forced t think there is some fundamental meaning in it. Even in the neural plate itse there may be a succession of axial determinations. There is a stage at whic hardly any transverse determination is present, although the antero-posterior ax

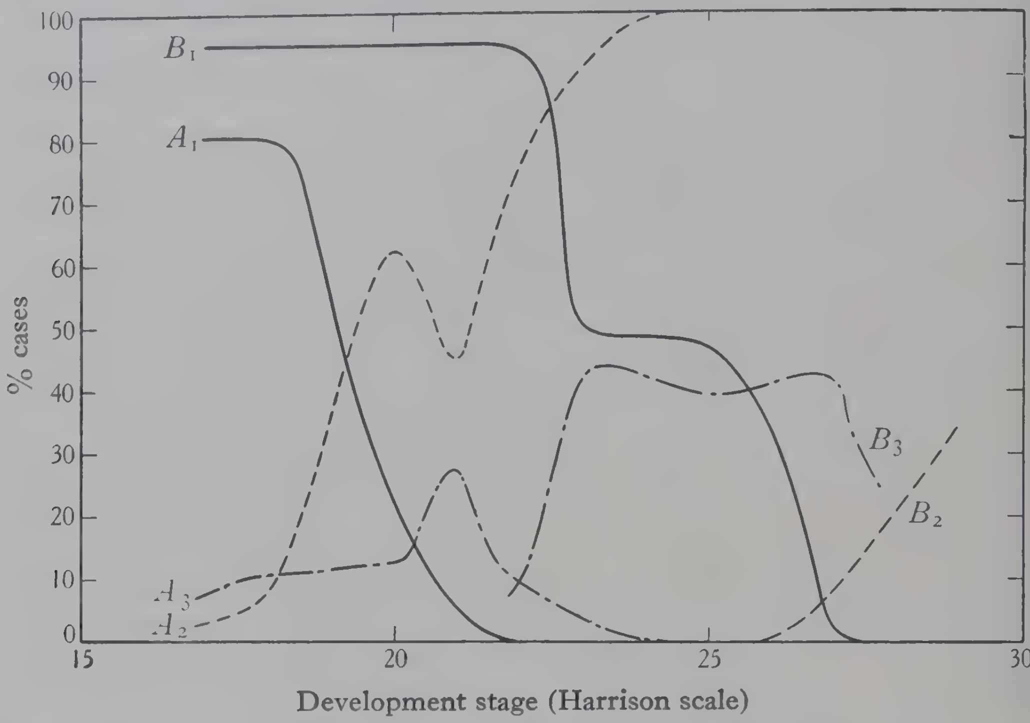

Fig. 326. Determination of spatial axes in the ear-vesicle of *Amblystoma*. (i) Antero-posterio axis only reversed. A_1, Ears with reversed asymmetry (neighbourwise; undetermined); A_2, ear with original asymmetry (selfwise; determined); A_3, ears showing reduplication anoma lies. (ii) Dorso-ventral axis only reversed. B_1, Ears with reversed asymmetry (neighbou wise; undetermined); B_2, ears with original asymmetry (selfwise; determined); B_3, ea showing vesicular anomalies.

quite fixed (Cummings). Similar considerations apply to the pronephros liment (T. C. Tung[3]).

A third point of great interest which arises out of Harrison's work on the ear hat reduplicated or enantiomorphic twin ear-vesicles sometimes appear. They ur statistically most frequently (up to 27 % of the total number of cases) very r the change-over point of antero-posterior determination (cf. Fig. 326). This is if the short transitional period was one in which the transplanted tissue uinely "did not know what to do". And correspondingly around the change- r point of dorso-ventral determination, curious vesicular anomalies occur st frequently.

'One is led from these facts", writes Harrison, "to the conclusion that the nsformation from the first to the second phase, and probably, in some measure east, from the second to the third phase, involve changes in the orientation of a-microscopic elements....With the newer knowledge of the crystalline icture of organic fibres in mind, it may not be too rash to expect that X-ray raction photographs may some day reveal the changes in finer structure which lerlie differentiations like those described as taking place in the development the ear." An attempt has been made (by Harrison, Astbury & Rudall) to nonstrate molecular orientation as the basis of axial determination, by direct ay photography of pieces of amphibian tissue, but the presence of water, k-material, etc. has unfortunately so far prevented any correlation. When it omes possible to cut sections sufficiently fine for the electron microscope, that l may have an important part to play in revealing the fine structure for which are looking. In any case, it remains a remarkable fact that just as simple dity in one, two or three dimensions occurs in liquid crystal states, so deter- nation (which is itself surely a very complex form of rigidity) in one, two or three nensions occurs in the organs of the developing embryo. It suggests that we have deal with an intra-cellular, but at the same time super-cellular, pattern ree dimensions, irreversibly polarisable along fairly independent axes. The amic structure in the cell, or "cell skeleton", would be involved in this.

omewhat analogous to the foregoing facts of organ development in amphibia the conclusions reached by Hörstadius[2] regarding determination of axes in eggs of invertebrates, especially the worm *Cerebratulus*. Here the animal- tal axis is determined between fertilisation and an early cleavage stage, while dorso-ventral axis is not determined till after the end of the cleavage stages. iversely, in the sea-urchin, the unfertilised egg already has a dorso-ventral , but the full determination of ectoderm and endoderm is not attained till the inning of gastrulation; hence animal-vegetal determination happens later than *erebratulus*. It will be remembered that dorso-ventral polarity can be shifted erimentally while the animal-vegetal cannot (in echinoderms, Runnström[2]; dahl[3]; in other invertebrates, Pease; but not in amphibia, Motomura[2]).

·55. Modifications of polarity by external influences

ne important property possessed by animal eggs is that their polarity is very d and almost impossible to alter. The only case in which the primary polarity of an animal egg has been determined or altered by centrifuging is that of C. V Taylor's work[2] on the gephyrean worm *Urechis*. With long-continued centrifugin (up to 18 hr.) at a force which would be regarded to-day as relatively low ($4800 \times g$ he was able to shift the primary egg axis, and the longer the centrifuging was continued the more the polar bodies tended to occur at the centrifugal pole. Bu these experiments have been criticised (Morgan & Tyler; Costello[2]) and appea to be of doubtful significance. Since the introduction of the air-turbine centrifuge, it has been possible to apply much greater centrifugal forces to cells (up to $800{,}000 \times g$), after which normal development has been shown to proceed (Costello[2] on nudibranch eggs; Beams & King[1] on *Ascaris* eggs; McDougald Beams & King on chick embryo heart fibroblasts). That cleavage polarity can be altered experimentally by pressure without producing any alteration in the primary embryonic axis is of course an old observation, known since Morgan's work[2] on *Arbacia* in 1893. Moreover, the dorso-ventral axis of echinoderm eggs is sensitive to external treatments, such as centrifuging, compression or local action of chemical substances (see pp. 497, 498).

It is obvious that when cell materials have been redistributed in the egg, the amount of actual ground substance in that part of the egg occupied by yolk-platelets or oil will be greatly reduced. Costello[1] has calculated that probably less than 25 % of the material in such a packed region is hyaline cytoplasm. The "cyto-skeleton", therefore, must be thought of, not as any series of protoplasmic strands, but as something molecular which can reform in time to permit of normal development. Hence the remark of F. R. Lillie[4] in 1909 is still relevant: "The existence of polarity and bilaterality in an optically homogeneous medium, and the persistence of both as to orientation under experimental conditions that seriously modify the quantitative relations of the oriented medium in different regions...seem to me to argue for a molecular basis of the fundamental principle of vital organisation."

It is interesting, however, that in plant "eggs" or spores the polarity is much more determinable by external influences. As long ago as 1885 it was noted by Stahl that differential exposure to light determined polarity in the spores of *Equisetum*. An interesting series of papers has been devoted by Whitaker to the eggs of the seaweed *Fucus*. The first division of these eggs gives rise to two cells of different shape, making the whole look like a pear. One, which includes the rhizoidal protuberance, is the parent cell for the formation of the rhizoid, the other gives rise by divisions to the thallus. The polarity of the eggs is directed by the presence of near-by neighbours, if more than a dozen are present, so that at the outskirts of a batch of eggs in a dish the pear-shaped embryos are all pointing inwards towards the main mass (Whitaker[2]). It was then found that the rhizoid protuberance always protrudes in the acid direction of a pH gradient (Whitaker[9]), unless the acid side is too acid, in which case complete reversal will occur and the protuberance will always point the other way (Whitaker[16]). This suggests that the mutual induction effect is due either to CO_2 or some other acid produced by the mass of cells, especially as the effect shows no species-specificity. If placed at one end of quartz capillary tubes, so that the gradient in one direction off more sharply than the other, the eggs will, when pear-shaped, point in the acid direction, i.e. down the capillary tube (Whitaker & Lowrance[2]). One esting effect is that when the sea-water is acidified to pH 6·0 all sizes gregations will carry out mutual inductions, and even two eggs in proximity send out the protuberance in each other's direction.* Temperature and electric ents are also effective; the rhizoid protuberance appearing always on the ned side (Lowrance) and on the side toward the positive pole (E. J. Lund[1]). s light. Diffuse white light speeds up the early development (Whitaker[10]) and cted white light (during a sensitive period) causes the rhizoid protuberance ppear on the dark side (Whitaker & Lowrance[1]). If the egg is deformed into gated shape by being sucked into a capillary tube, the rhizoid always forms ne of the ends (Whitaker[19]; cf. the determination of the ventral pole in echino- n eggs, p. 497). Finally, the polarity in this case can be affected by centri- ng, for Whitaker[12] found that the protuberance always arises at the centrifugal , away from the packed chloroplasts, though after redistribution of the rials this tendency disappears. This is true whether the centrifuging has been l $(3000 \times g)$ or severe $(150{,}000 \times g)$—(Whitaker[15]). But if the centrifuging ne in acid sea-water the polarity is 100 % reversed and the rhizoid pro- es at the centripetal pole[18].

f course the significance of these interesting observations for the problem olarity in the eggs of animals is not quite clear.† The protuberance of the oid seems to be due to a softening of the cellulose wall in that region, and the oid itself is sheathed with cellulose. After earlier failures, du Buy & Olson eeded in finding auxin in *Fucus* eggs, and it is therefore possible that all above facts may be explained by variations of the position of active auxin in the cell. This is supported by the occurrence of rhizoid protuberances at side of the eggs to which hetero-auxin is applied in sufficient concentration on & du Buy); but it does not help us in understanding polarity in animal ‡ In fact it reinforces the idea that in these we have to deal, not usually with novable polarity determiner, but with a fixed molecular three-dimensional dinate system, easily capable of being broken, but persistently re-forming .§

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is mutual effect has been analogised by Whitaker with the mutual effect of cohering echino- blastomeres in suppressing pluripotence. We thus have action only within the single cell ar inductors, pp. 408 ff.), then action across cell-boundaries (evocators, pp. 148 ff.) and action at a distance which may be ¼ mm.

ere is certainly a similarity between the directed appearance of the rhizoid protuberance the *Fucus* egg, and the directed appearance of the axon from the neuroblast in vertebrates, eiss points out. Moreover, the determination of polarity by electric currents is not utely unknown in animals. It is said that *Obelia* hydranths appear mainly at the anodic and stolons at the kathodic (E. J. Lund[2], confirmed by Barth[4]).

ight it be that the reason why the polarity of the plant egg is so readily altered, while that of nimal egg is so fixed, is because the relevant orienting micelles are in the former case wholly or y cellulose, and in the latter, protein?

the interesting paper of Pollister on the orientation of mitochondria in cells, seemingly ding to an invisible lattice of fibre-molecules.

3·56. Molecular orientation and contractility as the basis of sponse to morphogenetic inductors

Not only gastrulation, but neurulation also, must be thought of as involvi changes in the orientation of protein micro-fibres. It is a demonstration of o ignorance that although there has been so much to say in Section 2 about t stimulus of neural induction, so little has been said about the process itself, t intimate nature of that lengthening of cell and nucleus which constitutes t principal feature in the change from dorsal cuboidal ectoderm cell to neural tu cell. The only attempt to make some headway with this problem is contained the important but too much neglected papers of O. Glaser[2,3] (1914 and 191 and the abstract recognition of the problem of induction as one of molecul orientation by Rashevsky[1] (1932). During the folding for the neural plate to for the neural tube, the number of nuclei does not increase (confirmed later b Boerema), but there is a remarkable concentration of them in the half of t wall furthest from the lumen of the developing tube.* The main factor is th change in cell form, possibly due to extension of protein micro-fibres. There also a great increase in the volume of the neural area, as compared with th non-neural ectoderm. In the second paper, Glaser sought to correlate th vesiculation of the cephalic end of the neural tube with longitudinal compression, improving the old speculations of His on this matter.

It is possible that some light might be obtained on the way in which evocato substances affect the protein structure of the neural cells which they induce b recourse to surface film technique. When a naked blastula or gastrula is brough to an air-water surface, it immediately disintegrates, the formation of the powerfully spreading lipo-protein film being accompanied by a series of explosions easily visible under the dissecting microscope, as one cell after another cytolyses This bane of embryologists might be turned to good account if the effects o various substances, including evocators, were to be tested on the physicochemical properties of such monolayers, especially if films derived from ventra ectoderm and from dorsal blastopore lip or presumptive neural plate wer tested separately. The substances could be injected under the monolayers i the manner of Rideal & Schulman. Following this suggestion, a beginnin has been made by X-raying built-up newt embryo multilayers (Gatty & Schulman).

The probability is that molecular contractility plays a far greater part i development than embryologists have generally been willing to admit. Apar from the examples already given, we ought to remember the very extrem contractions of the chromosomes during mitosis, the blastodermal contraction of teleosts (p. 74), and in insect eggs the curious phenomena of blastokinesis or even more important, the wave of yolk contraction at the differentiation-centr (p. 461).

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* Later, as Sauer[1] has convincingly shown, the nuclei approach the lumen before dividing, an afterwards come away again. No explanation is forthcoming for this behaviour.

57. Anisometric protein molecules in eggs and embryos

nother way of getting some light on the problem would be to ask whether the ecular shape of the protein molecules of the egg hyaloplasm is globular (as iratory pigment molecules, for instance, seem to be) or fibrillar. If the latter e true, it would be easier to imagine the dynamic lattice of protein chains. esides the protein of muscle, many other groups of highly anisometric ecules in the body are now known, e.g. the stroma protein of erythrocytes hm), where the molecular length is 1000 times the breadth. The most direct hod would be to prepare the protein of "yolkless" eggs (such as those of urchins) and examine it, either in the ultra-centrifuge or with co-axial ting cylinders. v. Muralt & Edsall made such a study (now classical) of the perties of muscle globulin (myosin). Having separated it with the utmost care n the other constituents of muscle, they enclosed it in a space between two centric cylinders, one of which revolves so that the shear force orients particles in a uniform direction. Then, observing the flow birefringence ugh crossed Nicol prisms in a direction longitudinal to the cylinders, found the "cross of isocline". This optical phenomenon means that the ticles are all *rods* of nearly the same size, identical, in fact, with the crystals viously postulated to account for the double refraction of the muscle fibre. ilar work upon egg-proteins has already begun.

efore describing it, we may recall that all eggs appear to contain two main sses of protein (p. 15). On the one hand there are the vitellins or ichthulins, sphoproteins containing much serine phosphoric acid; on the other hand there the livetins or thuicthins, which are pseudo-globulins not unrelated to the osin of muscle. It might be that the former are primarily stores of nitrogen l phosphorus for the subsequent elaboration of the embryo's architecture, and t the latter (much less usually in amount) are the molecules constituting the o-skeleton".

the first place direct observation of echinoderm eggs has succeeded in aling phases of birefringence within the cell (W. J. Schmidt[4]; Moore & er). Pfeiffer[3] submitted mollusc eggs to deformation within capillary tubes, described interference striations from which the existence of fibrillar ecules was deduced. Longitudinal striations have also been reported by riz[2] in living amoeboid protoplasm by the aid of the Spierer oil-immersion -ground ultra-microscope. Mirsky's examination[2] of echinoderm egg-proteins re and after fertilisation is also rather significant. He brought about the ntegration of the cell structure by freezing the eggs in solid CO_2 before ing them in a high-vacuum desiccator; by this method (believed to distort protein molecules less than any other), he found distinctly more of the ble protein fraction before fertilisation than afterwards. The protein that gulates during fertilisation he showed to consist of very fibrillar molecules, it had a high viscosity and showed flow birefringence.

ome work has also been done on the hen's egg. Böhm & Signer found a very ked flow birefringence in ovoglobulin preparations (probably mixtures of ovomucin and ovomucoid) but none in ovoalbumen. Wöhlisch & Belonoschkin also found that ovoglobulin gave a value for the Gans effect (depolarisation of the Tyndall beam) higher even than myosin itself, again indicating the presence of very long molecules. As the yolk might be regarded as of greater embryological significance than the white, Needham & Robinson examined livetin preparations in an improved co-axial cylinder apparatus also capable of acting as a Couette viscosimeter* (Robinson[1]; Lawrence & Robinson). They found flow birefringence though weaker than that of myosin, and an accompanying thixotropy and rheopexy. Vitellin and vitellomucoid did not show these properties. However, when this work was continued by Lawrence, Needham & Shen with an improved Couette apparatus on amphibian material, the total globulin fraction (containing vitellin) showed regular anomalous viscosity at low rates of shear, closely resembling that of tobacco-mosaic virus. The pseudo-globulin, on the other hand, though showing at first normal flow, formed polyfilms at the air-water interface after some rotation from which particles giving anomalous flow appeared to be split off. Much further work is needed along these lines. For example, the ultracentrifuge may be expected to permit the separation of the protein fractions containing anisometric molecules.

It must also be significant that the particles of many plant viruses are highly fibrillar (X-ray evidence of Bawden, Pirie, Bernal & Fankuchen; viscosity and flow birefringence evidence of Robinson[2]). W. M. Stanley emphasises the importance of this as linking chemical with morphological forms, but it has not yet been possible to form a picture of exactly how the fibrillar molecules are fitted together in the cell. Since the viruses are nucleoproteins, the suggestion that chromosomes are paracrystalline protein macro-molecules, is of great interest (see p. 637). It was first made by Koltzov[1] in 1912 with nothing more to go upon than the polypeptide chains of Emil Fischer, and since that time the proposal has been developed with new evidence in a remarkable way (Wrinch[2]; Koltzov[2,3,4]).

Less important embryologically, but still not without interest, are the observations of W. J. Schmidt[3] on the complex crystal structure of *Ascaris* egg-shells, in which chitin fibrils are involved.

One cannot leave the subject of fibres and micro-fibres without referring to a type of living matter at first sight somewhat far removed from the animal embryo — the plasmodium or slime-mould. According to a remarkable observation of Arthur Lister in 1888 such a mass of protoplasm will flow through cotton-wool: "I placed some wet cotton-wool in front of the still dingy plasmodium; this it readily penetrated, and afterwards emerged possessing its normal yellow colour, leaving the wool charged with spores and other debris." Recently Moore[4] has pushed further the analysis of protoplasmic structure along these lines. *Physarum polycephalum*, a slime-mould, was not killed by being ground in a mortar with quartz sand, but was killed if finely crushed glass was used instead. Both soft and hard filter papers permitted the passage of the plasmodium, and it readily passed through parchment, where the pores were of an average diameter

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* The inner cylinder is supported on a torsion wire, and the deflection at different speeds read off with a mirror.

10^{-5} mm. Yet if pressure was applied, as by forcing it through textiles or paper, the protoplasm was destroyed at far larger pore sizes. Thus to be zed through fine silk (pore size, 5×10^{-2} mm.) was lethal for it, and pores ge as 0·25 mm. had to be used before it could be safely pressed through. se results suggest", says Moore, "that the plasmodium contains long ds of living material essential to its existence, and if these be broken too , life is impossible." From the rough data just given such micro-fibrils l be 5×10^{-5} mm. in diameter and 2000 times as long, i.e. about 100 times iameter of the protein chains envisaged by Peters. However, the general stiveness of these experiments cannot be questioned.

subsequent papers Moore[5,6] tells us that filtration treatments which are lethal nost lethal for the plasmodium reduce its respiratory rate 50 %. Severe y may also be caused by centrifuging at 75,000 × *g* for a few minutes.

58. The mechanism of gastrulation

at embryology may become largely the study of protein fibres and their ed distortion and orientation may be illustrated by further examples. erly there was much discussion about what was called the inadequacy of ell theory of development (Sedgwick; Whitman). But whereas this mainly d on the meaning of the histological evidence, i.e. the existence and nature ll boundaries, in our day the importance of the cell has largely evaporated, se we tend to think more in terms of embryonic *regions*. Determination ates, as far as individual cells are concerned, in a statistical way. Whether n cell is just inside or just outside the boundary of a field or district influence t a matter of much importance. But recently the question has again been ght to the fore by the experimental demonstration of the existence of cell ges in the early development of echinoderm eggs (A. R. Moore[1]; M. M. re; Moore & Moore; Huang) and the suggestion that these may have tion of some importance in gastrulation. Here is a fundamental field for plication of X-ray analysis. It might be well worth while to know something crystal structure of the protein chains of these cell bridges. Is it not cant, in view of what we now know of molecular contractility, that they ss considerable elasticity?

ong and interesting chapter in embryology has been made by efforts to stand the process of gastrulation. Forty years ago Rhumbler[2] regarded ination as being due to a change of shape in the cells of the vegetal pole of lastula so that their internal bases were broadened, but in most cases no shape has ever been observed. Then Bütschli thought that differential ption of water might be responsible, but Moore's analysis of lithium exo- ulation[2] has fairly clearly disproved this possibility. Moore considers eton's suggestion[2] more hopeful, namely, that if the cells of the blastula ct each other and if the centres of attraction are eccentric, any increase in ure will result in an invagination if the centres are peripheral or an evagina- f the centres are located in the basal parts of the cells. The nuclei themselves cannot be, as Assheton thought, these centres of attraction, since in most echi derms, for example, they are disposed towards the periphery at both poles of blastula, i.e. at the presumptive non-invaginating part as well as at the p sumptive site of invagination. Moore's study[1] of cell bridges by micro-dissecti however, has shown that they might perform this function. They certainly se to account for the tendency to curl at the edges, which cell plates (e.g. in *Dendra* developing without the fertilisation membrane) exhibit.

Nothing quite similar to this has been found in amphibian embryos, Waddington's examination[24] of the forces involved in gastrulation, though o preliminary, shows the way for further work. The invaginating mesoderm made to act upon a steel ball subject to the opposing force of a magnet; in t way it was found that the invaginating material could successfully oppose a fo of 0·34 mgm./sq. mm. Another point to be remembered is the suspicion alrea referred to (p. 146) that the initiation of gastrulation, at any rate, is connect with contractility on the part of the piriform cells of the endoderm. Holtfreter too, has studied the natural curling propensities of isolated pieces of the amphib gastrula and neurula. The neural plate curls in concave fashion, the epiderm convex. Speed and intensity of curling are sensitive to osmotic pressure, and t curling tendency is present before it occurs in the normal course of developmen

The subject has been pursued by Moore & Burt, who report that the invagin tion tendency of the echinoderm gastrula is inherent in the gastral plate. If t gastral plates are excised from sea-urchin embryos, the edges curl up and m eventually meet and fuse, but nevertheless invagination continues. If there not enough ectodermal material for this, the gastral plate will eventually for a naked endodermal sphere. These facts show that the invagination tendenci are intrinsic to the invaginating area. Other experiments of Moore & Bu indicate that the composition of the blastocoele fluid can have little to do wi the process because it is almost identical with sea-water. The only changes in concern the *p*H, but this is not till after gastrulation. At first more acid than t environmental sea-water, it rises when the mesenchyme cells first appear to level above that of sea-water (Hirabayashi; in confirmation of Rapkine & Prenan not of Chambers & Pollack; see *CE*, p. 847). If the pressure of the blastocoele flu is raised to a sufficient degree (as by the use of sucrose-containing sea-wate gastrulation may be delicately inhibited (Moore[8]).

3·59. The fibres of the mesoderm

We come lastly to the fibrils of connective tissue ground substance. It has lo been known that the organs first formed in the embryo (neural tube, notochor somites, etc.) are held apart by a "ground substance", the precursor of connecti tissue, which at first contains no cells. This has long been known and oft observed and figured, as by Merkel; von Szily; and in the beautiful series papers by Baitsell. It has been suggested (*CE*, p. 566) that this material, whi histochemically contains a high proportion of mucoprotein (Bianchi), and whi occurs in relatively largest amount in the earlier stages of development, accour for the high level of protein-combined carbohydrate found during amphibi

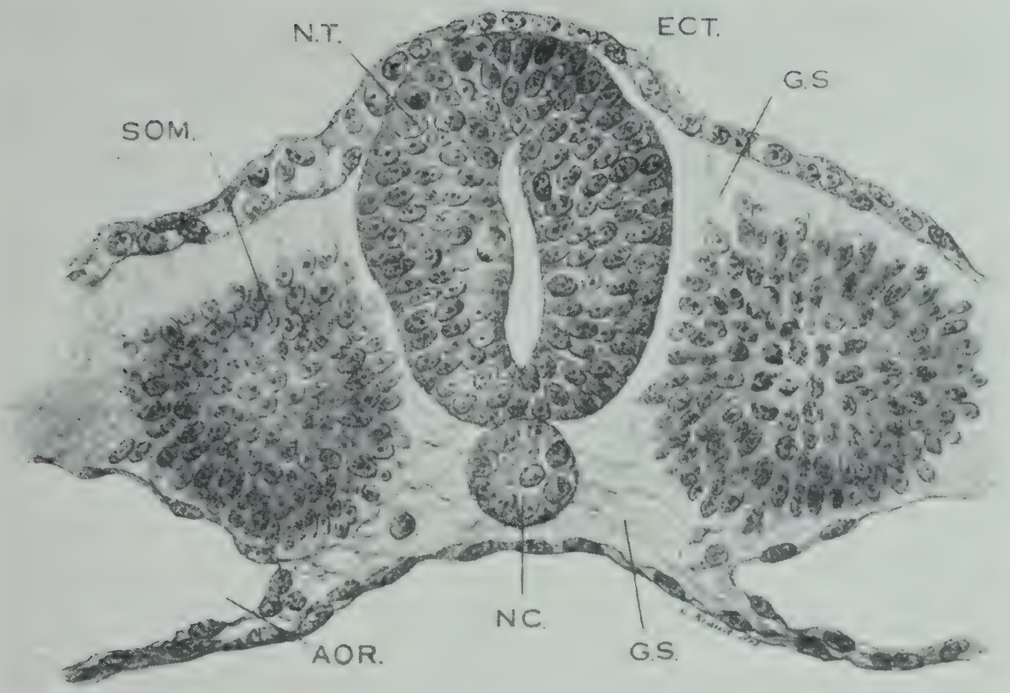

Fig. 327. Connective tissue ground substance fibrils in the early chick embryo. *AOR*, Aorta; *SOM*, somites; *NC*, notochord; *NT*, neural tube; *ECT*, ectoderm; *GS*, ground substance.

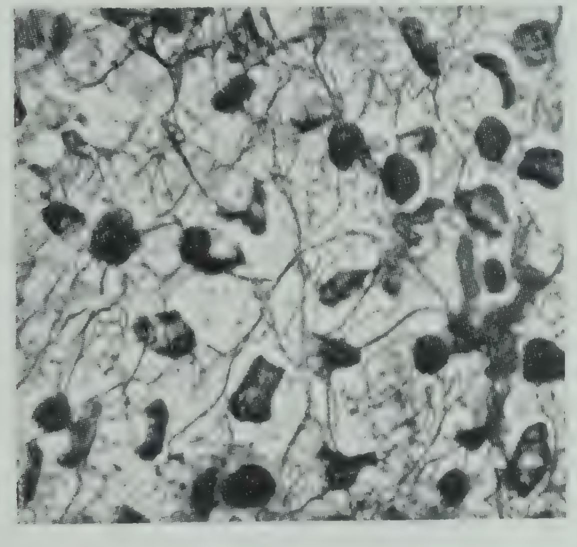

Fig. 328. Primitive connective-tissue fibrils of the exocoelom of the chick embryo.

ɪpment. The important point is that wavy fibrils appear, as if by a sort of .llisation, in this ground substance some considerable time before any cells are ıt in it (Fig. 327). Cells migrate into it later (Baitsell[4,5]; Bauer). Analogous sses have been shown to be at work in the development of clot material g wound healing (Baitsell[2]), and in plasma clots subjected to traction : there seems to be a continuity between fibrin filaments and the later (Baitsell[3]),* and also in the origin of fibrous tissue arising at sites of tu- lous infection (Baitsell[6]; Baitsell & Mason). The "crystallisation" of netric protein molecules must certainly be involved in these processes. 28, taken from Snessarev's review, shows a micro-photograph of the outer of the exocoelom of a chick embryo of 72 hr. incubation; the primitive ective-tissue fibrils can be clearly seen. The appearance of the wavy fibrils in cultures has been also studied a good deal (Baitsell[1]; Rumjantzev & :ova; Berezkina; Litvać). The observation of Berezkina that the old fibres as a basis for the new ones suggests a crystallisation process originating "nuclei" provided. If the ground substance is a secretion of the embryonic as it must be, it should be possible to detect some cytological evidence of hence the interest of Cameron's contention that the Eberth bodies of ibian embryonic epidermis are concerned in the secretion of ground ance. Furthermore, Litvać has shown that the fibrils formed in the ıd substance of tissue cultures are birefringent, and hence probably para- ılline in nature.

e subject has been much amplified from the experimental angle by Weiss[9], first showed that if a membrane of blood plasma is subjected to mechanical ɔn, e.g. by placing it in frames of different geometrical forms, then fibro- cultures growing upon it will show an oriented migration directed along ain axes of tension.† This is interpreted to mean (Weiss[12]) that the micelles rillar protein molecules of the substratum are oriented, and that the cells and migrate accordingly. Similar results were obtained by Huzella. Later [13] extended these ideas to the growth of nerve fibres, which probably occurs as the result of following the fibrillar ultra-structure than on account of her factors.

while we cannot fail to admit the importance of ultra-structure in guiding ıogenetic processes, we are forced back on the mystery of how the ultra- ure itself is formed. Probably the only way in which we can think of this ı kind of extension of the pattern ("dynamic structure") possessed by the ɔf the primitive organs themselves. We may recall W. B. Hardy's theory[1,2] phic action, which still remains, indeed, the only attempt to explain that ıs phenomenon. Tonus and heat production of muscles depend on con- :y of nerve-supply, but it is not believed that the nerve fibre here acts by :ying impulses to the muscle. Hardy[2] suggested that its effect might be due

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s generally agreed, however, that fibrin is by no means *necessary* as a precursor of fibril ion.

exact physical nature and origin of this micellar orientation is a matter of discussion (see te of Pfeiffer[2]).

to orientation of molecules. A layer of molecules oriented by an interface mi he thought, be of the order of at least a thousand molecules in thickness. Th he wrote, would be a humble example to place beside the colossal examp furnished by living matter, "but when one has for years contemplated a scienti problem towards the explanation of which nothing could be advanced, even t slightest clue is welcome".

This suggestion derived from the work of Hardy & Nottage in which it w shown that orientation effects may be transmitted from a surface such as th of a metal capable of influencing a surrounding film for a distance of 7 to 8 "Here we have at once", wrote Peters, commenting upon this later, "a means which a mosaic may radiate its effects throughout the cell. It is possible appreciate how a co-ordinate structure may be maintained even in a mediu which is apparently entirely liquid. We have in effect chains of liquid crysta which need not be more than a few molecules thick. Once we have seen thi it becomes possible to develop some dim conception as to how the miracle occur that colloid molecules, which have apparently no particular location in the ce can yet control the synthetic and other reactions which are proceeding. Thi theory also is all that is needed to enable us to understand how substances ca reach a special site in the cell. Between the chains of molecules, fixed by th radiating webs, there will exist paths from the external to the internal surface."

Related to the connective-tissue fibrils mentioned above are the collagen or elastoidin fibres of tendons, fins, etc., which show birefringence and are certainly built up of anisometric molecules. These have been much studied by Fauré-Fremiet and his school (Fauré-Fremiet[5,6]; Fauré-Fremiet & Garrault[2]; Fauré-Fremiet & Baudouy[1]; Fauré-Fremiet & Woelfflin; Garrault[2]) and X-ray photographs have been made (Champetier & Fauré-Fremiet). Ingenious methods of micro-dissection using protozoa which do not attack the fibres have been used (Fauré-Fremiet, Kruszynski & Mazoué).

We do not yet know, of course, the normal equilibrium configuration of most of these fibres. We could guess, however, that the Weissian connective-tissue ground substance fibres are usually contracted, like the chains of wool, while the cell bridges of echinoderms and the yolk fibres of insects are usually expanded like the chains of silk. On such a view, the elongation of the cells during neura plate formation might be connected with the expansion of molecules previously contracted, and the clamping of them in their new condition. But that is as far as speculation can well go in the present state of our knowledge.

3·595. Dynamic structure at the molecular level

Throughout this book in general, and the foregoing section in particular, it has been necessary to emphasise our reliance upon the proteins as the responsible structures in competence (pp. 114, 119, 203), cell-architecture (p. 670), dynamic structure (p. 658), and so on. But it would be a great mistake to think of them as static solid material like that which is used by the human architect or statuary The protein molecule in the living body, though not when isolated and purified is in a condition of constant flux and flow, and if it participates in living itecture, it participates in metabolism too. This had indeed long been known, the extraordinary speed at which the replacement of atoms in molecules in the living cell occurs was not revealed until the employment of radio- e and other isotopes permitted the use of marked or tagged atoms (reviews choenheimer & Rittenberg[2]; Hevesy; van Heyningen[2] and others). Hence the ein molecule itself, no less than the cell-structure of which it is a part, is a rn the components of which are in perpetual motion. In visualising this, it ifficult not to think of it as a prefiguration of the mutual collaboration of al units in maintaining patterns at far higher levels of organisation.

CONCLUSION

he foregoing sections, which conclude the plan of the present work, have hasised successfully, one may hope, that the polarity and symmetry pro- ies of embryos will never be understood except in the light of a knowledge ne dynamic structure of the egg-cell. The problem of these properties has us directly to the fundamental problem of biology, the nature of cytological morphological organisation. The whole object of the book was, after all, to v that the fields of chemistry and morphology are not so sundered as is n supposed. Organising relations are found at the molecular level and at the idal and paracrystalline level, the level of protein macro-molecules and highly merised substances, just as clearly as at the anatomical level itself. Although re still in the earliest stages of any real theory of living organisation, we can see that biological order, like crystal order, but on a much more complicated e, is a natural consequence of the properties of matter, and one characteristic e of their manifestation. But when this is said and accepted, the task of ryologists is far from being on the point of ending. On the contrary, only , freed from the vestiges of primitive philosophy, can they look forward to their real task clearly in the future, the revealing of the causal sequence reby the organisation of the fully formed organism arises from the lesser nisation of its zygote. Human science is yet young and the task of embryo- sts is really only beginning. Human society itself, in that slow but persistent inevitable rise in level of organisation which biological and social evolution esents, mirrors, as the great Victorian biologists recognised very well, the genetic rise of the individual organism in organisational level. Let us hope human society will before long attain a level of organisation such as to urage and facilitate the great enterprise of embryology. The study of eginnings of life has something in it antithetical to death, the harmonious ions of morphogenesis something inimical to human disunity and hatred, co-operation of embryonic processes something utterly alien to human ession and war. But we are not as men without hope, for just as the greater ber of ontogenies safely achieve their triumphant ends, so biological and l evolution, in spite of many grave setbacks, give us the right to look forward confidence to the *Regnum Dei* of man's full stature.

GLOSSARY OF SOME OF THE SPECIAL TERMS USED IN THIS WORK

(Cf. the "Terminologie" of Roux, Correns, Fischer & Küster)

ivation. The liberation of naturally ccurring evocators from inactive combina- on. *See also* Fertilisation.

ivation centre (*Bildungszentrum*). One of ne two organisation centres in the insect egg ee p. 460).

inity. The extent to which tissues from arly embryos tend to cling together when in olation from their normal surroundings; Roux's Cytarme.

cithic. Having little yolk; said of e.g. chinoderm eggs.

antois. The sac developing from the hind- ut in amniotes, which receives the foetal xcretions, and, in birds and some mammals, as important respiratory and nutritional nctions.

ometry. Allomorphosis and Heterauxesis.

omorphosis. The relation of parts of rganisms at some definite age to wholes or arts also at some definite age, but of different roups (races, varieties, species, genera); e.g. gg size or hatching weight to adult size or eight.

nion. The sac filled with liquid immedi- tely surrounding the embryo.

alogous. Said of organs or structures hich have the same function but are of ifferent evolutionary origin. *See* Homo- gous.

rogen. A chemical substance with ac- ity similar to that of the male sex rmones.

rogenesis. The fertilisation of an egg, the cleus of which has been so treated that clear fusion cannot ensue, by normal erm.

ro-merogony. *See* Merogony.

dian. A blastoderm on which no trace of nbryonic axis appears.

mal pole. That pole of the egg which ontains most cytoplasm and least yolk.

malisation (*Ectodermisation*). The shifting f the presumptive fate of tissue which hould have been endodermal (in echino- erm development).

lage (*Rudiment*, *Ébauche*). A group of cells, ot necessarily distinguishable from their urroundings, which will later give rise to a iven organ.

a. A morphogenetic area is one of the onstituent regions on a Fate-Map.

Area opaca. The opaque yolky extra-embryonic blastoderm of the sauropsids, which later forms the yolk-sac.

Area pellucida. The transparent central part of the sauropsidan blastoderm, from which the axiated embryo arises.

Auflagerung. The laying of competent ectoderm upon a dead inductor in order to test inductive power.

Autonomous induction. An induction in which the inductor takes no part itself in what is formed (opp. of *Complementary induction*). All chemical inductions are autonomous inductions.

Autoplastic transplantation. Transplantation between different sites on the same individual.

Autotomy. The spontaneous shedding of an appendage (arthropods).

Auxesis. Growth in size by cell expansion without cell-division.

Bauchstück. The ventral half of an amphibian gastrula, from which all the organiser region has been removed; hence it never forms any neural axis.

Bedeutungsfremde Selbstdifferenzierung. Self-differentiation in accordance neither with the tissue's original presumptive fate nor with the presumptive fate implied by the new surroundings, i.e. neither selfwise nor neighbourwise.

Bildungszentrum. *See* Activation centre.

Blastema. The small bud composed of competent cells from which begins the regeneration of an organ or appendage.

Blastocoele. The cavity in the blastula.

Blastocyst. The mammalian embryo at the time of implantation into the uterine wall.

Blastoderm. The flat sheet formed by the embryo's cell-layers when cleavage is discoidal.

Blastokinesis (*Revolution*). A process of cephalo-caudal reversal in the egg, accomplished in some animals by movement during development (e.g. insects).

Blastomere. One of the cells into which the egg divides during cleavage; the term is not usually applied after the blastula stage. When blastomeres or sets of blastomeres differ in size, the terms *macromere* and *micromere* are used.

Blastula. The stage in development at which a central cavity is formed in the mass of dividing cells (the *Blastocoele* cavity).

Bradyauxesis. *See* Heterauxesis.

Carcinogen. A chemical substance which has the property of giving rise to cancer when administered to animals by external or internal application.

Cecidogen. A chemical substance, usually secreted by an insect, which has the property of giving rise to a gall on a plant.

Centrolecithal. Having yolk accumulated at the centre of the egg (e.g. arthropods).

Chimaera. A compound organism or tissue obtained by grafting or other means so that it is composed of tissues of different kinds (e.g. an andro-merogonic head on a normal diploid tail) or of different species or genera.

Chorio-Allantoic Grafting. The placing of small pieces of avian or mammalian embryos upon the chick embryo's allantois under the inner shell-membrane (the bird's egg has no chorion), in which position they become vascularised from the allantoic circulation and develop, though not in a strictly neutral medium.

Cleavage. The processes of cell-division by which the original egg-cell gives rise to all the cells of the organism; if the cells are from the beginning of approximately equivalent size, the cleavage is *holoblastic*; if some are much larger than others owing to yolk accumulation or other reasons, the cleavage is *meroblastic*; and if cell-division occurs only at the surface of an enormous yolk-mass, the cleavage is *discoidal*. Cleavage in some species follows a definite pattern, where it is said to be *determinate* (this has permitted the tracing of "*cell lineages*"); in other species the pattern is quite lost after the first few cell-divisions.

Cleidoic. Highly isolated from the environment, e.g. the eggs of birds, with walls permeable only to matter in the gaseous state.

Colostrum. The first secretion from the mammalian mammary glands after the birth of young; it has quite different properties from the milk which it precedes.

Competence. The state of reactivity of a part of an embryo, enabling it to react to a given morphogenetic stimulus by determination and differentiation in a given direction. An embryonic cell develops as many competences as it has prospective potencies or possible morphogenetic fates. Generally speaking, determination to proceed to one morphogenetic destination cancels the other competences previously possessed by the cell. Competences appear in a succession at definite stages of development and later disappear, whether or not any of them have been implemented by the appropriate morphogenetic stimulus. Some comp tences exist in the adult tissues, if we m so term the states of reactivity to hormo action, though these effects are general reversible (cf. *Modulation*).

Complementary induction. An induction i which the inductor itself is built into th morphological complex which it has induced (opp. of *Autonomous induction*).

Compression. The acceleration of development or extension of the period befor hatching, so that the larval stages ar accomplished or omitted before the organism hatches from the egg.

Corporative. Differentiation due to th physiological functioning of parts, as opposed to *Individuative*, due to the action o morphogenetic fields.

Cyclopia. The development of one media eye instead of two lateral ones.

Cytotaxis. The spontaneous attractive o repulsive movements of cell-groups. *Se also* Affinity *and* Incompatibility.

Dedifferentiation (*Einschmelzung*, *Catachony*). Loss of differentiation, as of organelles by protozoa before cleavage, or of starving ascidians, or of the vertebrate limb stump in blastema formation.

Degrowth. Decrease in spatial dimensions and weight of an organism.

Dependent differentiation (*Correlative or abhängige Differenzierung*; *Différentiation provoquée*). The differentiation of a tissue partly as a response to factors existing outside itself, i.e. differentiation which requires the morphogenetic stimulus of an inductor from a neighbouring part of the embryo.

Determination. The fixation of the fate of a part of an embryo, so that (if it develops further) it will perform one kind of morpho- or histo-genesis, and one kind only, irrespective of the situation in which it may find itself. In using this concept one must bear in mind that all possible situations have usually not been tested; hence the word must be handled with due regard to the limitations of our knowledge. Ideally, the situations should be specified in every case, but definition will continue to be the victim of convenience until some rapid notation is devised. The term should not be used for the status of the tissue following the process, and it should have reference to an immediately subsequent developmental change. It must be remembered that determination is embryological, not genetical. This is seen with special force when ontogenetic stimulus-response systems are considered in which the stimulator and the reactor are of genetically different tissue. The sense in which the word "determination" is here used must be distinguished from other uses, e.g. (1) the "determination" of the original axes of the

ermination (*cont.*)
g by external factors (Roux), (2) the "deter-
inate" cleavage of eggs in which the cleavage
attern is very definite and permits of accurate
escriptions and predictions of cell lineage
Conklin); (3) "determinants", hypothetical
nits in the germ-plasm (Weismann), a use
ow obsolete. *See also* Dynamic determina-
on, Material determination.

pause. A state of dormancy in the normal
evelopment of numerous animals, especi-
ly insects, and distinguished from hiberna-
on in that it must be passed through, no
atter what the temperature or other
ternal conditions may be.

ferentiation. Increase in complexity and
rganisation. Increase in number of kinds of
ells; invisibly, as by determination and
sses of competence; visibly, as by histo-
enesis. Increase in morphological hetero-
eneity by the appearance of form and
attern. *See also* Self-differentiation, De-
endent differentiation (Correlative differ-
ntiation), Functional differentiation (Cor-
orative differentiation), Regional differenti-
tion.

ferentiation centre (*Differenzierungszen-*
um). One of the two organisation centres
the insect egg (see p. 460).

loid. Containing the normal number of
hromosomes.

ect induction. The induction of a new
eural axis in competent ventral ectoderm
y a chemical compound acting in the same
ay as the naturally occurring evocator.

trict (*Territoire*). A minor morphogenetic
eld, possessing a general determination for
roduction of a certain structure or organ, and
ndergoing progressive regional specification
f detail, e.g. limb district or gill district.

ble assurance (*doppelte Sicherung*). The
ility of an embryo to perform a morpho-
netic process by a method not normally
ployed, if the usual method is inhibited
experimental means (Spemann). Reasons
e given on pp. 157, 294, 308 for believing
at this is not a true category of morpho-
netic behaviour. The normal concurrence
many factors towards the production of a
ven morphogenetic result may, however,
admitted (cf. the *synergetical principle*
development, the *kombinative Einheits-*
istung of Lehmann).

amic determination. Determination of
part of an embryo to carry out a certain set
f morphogenetic movements.

oderm. The outermost of the three cell- or
erm-layers of classical embryology, see
ig. 55. Strictly speaking, the term ecto-
erm should not be used until the definitive
toderm and mesoderm have fully formed,
ut the loose usage avoids multiplicity of
rms.

Eidogen. A chemical substance which has the power of modifying the form of an embryonic organ which has been induced by other means. The term is intended to cover such substances as may be involved, beside the primary evocator, in bringing about regional differentiation of the neural axis; but it also would be applicable to such substances as are involved in *Nachbarschaft* or *Contiguity* effects, including those which diffuse from diploid into haploid tissue (cf. p. 360), and it grades, without a very sharp dividing line, into the category of the second-grade inductor.

Einsteckung. The implantation of a tissue, living or dead, or a piece of implant medium containing one or more chemical substances, into the blastocoele cavity of the gastrula, in order to test induction power.

Emancipation. The process whereby the various organ districts acquire their boundaries in arising out of the primitive individuation field.

Embryoma. *See* Teratoma.

Endoderm. The innermost of the three cell- or germ-layers of classical embryology.

Epiboly. The expansion of the ectoderm to surround the endoderm and mesoderm, whether by their invagination or its own active envelopment of them.

Epigenesis. The development of the organism by the new appearance of structures and functions, as against the unfolding or growth of entities already fully present in the egg at the beginning of development (*Preformation*).

Evocation. That part of the morphogenetic effect of an organiser which can be referred back to the action of a single chemical substance, the *Evocator*.

Evocator. The chemical substance, acting as the whole or part of a morphogenetic stimulus, emitted by an organiser.

Exogastrulation. Formation of a Teras by gastrular evagination instead of the normal invagination.

Explantation. The development and growth of cells outside the organism from which they were derived, and in isolation from other tissue, i.e. *in vitro*.

Expressivity. The extent to which an animal carrying a gene and showing it is affected by it (the relative severity of the effect). *See also* Penetrance.

Fate-map. A map of an embryo in an early stage of development, e.g. the late blastula stage, indicating the various regions whose prospective significance has been established by marking methods. The regions are known as *Areas*, e.g. the area of presumptive notochord material.

Fertilisation. The essential stimulus to development given by the spermatozoon to

Fertilisation (*cont.*)
the egg. In artificial *Parthenogenesis* this stimulus may be replaced by a wide variety of agents, some of which do not effect more than a few of the preliminary cytological changes preceding cleavage; this is known as *Activation*; others stimulate more profoundly so as to put in motion the cleavage processes (*Regulation*).

Field. A morphogenetic field is a system of order such that the positions taken up by unstable entities in one part of the system bear a definite relation to the positions taken up by other unstable entities in other parts of the system. The field effect is constituted by their several equilibrium positions. A field is bound to a material substratum from which a dynamic pattern arises. It is heteroaxial and heteropolar, has recognisably separate districts, and can, like a magnetic field, maintain its pattern when its mass is either reduced or increased. It can fuse with a similar pattern entering with new material if the axial orientation is favourable. The morphogenetic *gradient* is a special limited case of the morphogenetic field.

Foetus. Medical synonym for embryo.

Freemartin. A mammalian intersex due to the masculinisation of a female twin by its male partner when the foetal circulations are continuous.

Functional differentiation. The differentiation of tissues as the result of forces arising out of the functions which they are performing.

Gastrulation. The processes whereby the endoderm, mesoderm, and ectoderm come to occupy their eventual position in the embryo.

Genotype. The genetic equipment of an organism.

Gestalt. A patterned whole distinguished from its surroundings.

Grade. *See* Organiser. Organisers are said to be of first-, second-, or third-grade according to the time, whether early or late, at which they act during development.

Gradient. A system of order involving progressively increasing or decreasing entities or intensities from one pole or point of a morphogenetic field to another. It is a two-dimensional special case of field pattern. *See* Field.

Growth. Increase in spatial dimensions and weight of the organism. Increase in number of nuclei and cells (*Multiplicative* or *Meristic growth*). Increase of cell-size (*Auxetic growth*). Increase of non-living structural matter (*Accretionary growth*). The increase of living respiring protoplasm at the expense of inert yolk during embryonic development in holoblastic eggs should probably be regarded as growth although the total weight of the system may be decreasin owing to loss of water and carbon dioxide i combustion of energy sources.

Gynandromorph. A sex-mosaic. *See* Mosai (2). Contrast with *Intersex*, all of whose cel have a genetic constitution intermedia between male and female.

Gynogenesis. The fertilisation of an egg b sperm so treated that nuclear fusion canno ensue.

Gyno-merogony. *See* Merogony.

Haemotrophe. The sum total of nutritiv substances supplied to the embryo from the maternal blood stream in viviparous animals.

Haploid. Containing half the normal number of chromosomes, i.e. one of each of the necessary kinds.

Herkunftsgemäss. *See* Selfwise.

Heterauxesis. The relation of the growth rate of a part of the developing organism (whether morphological or chemical) to th growth rate of the whole, or of another part. The part may grow more rapidly than the whole (*Tachyauxesis*) or it may grow more slowly than the whole (*Bradyauxesis*), or at the same rate (*Isauxesis*). Heterauxesis is a comparison between organisms of the same group but of different ages and hence sizes. *See also* Auxesis, Merisis, Allometry, Allomorphosis.

Heterogametic sex. That sex the gametes of which are divided into two populations, one of which possesses the X-chromosome, governing sex differentiation, and the other does not.

Heteromorphosis. The appearance, whether in embryonic development or in regeneration, of an organ or appendage inappropriate to its site, e.g. antenna instead of leg or eye. Embryonic heteromorphosis was termed by Bateson *Homoeosis*; Goethe knew it as *Metamorphy*.

Heteroplastic transplantation. Transplantation between individuals of different species within the same genus.

Heterotopic transplantation. Transplantation between different sites.

Hippomanes. Packets of uterine secretion surrounded by trophoblast and invaginated into the allantoic cavity.

Histogenesis (*Elaboration*). The development of histological differentiation.

Histolysis. Tissue destruction, as in insect metamorphosis.

Histotrophe. The sum total of nutritive substances supplied to the embryo in viviparous animals from sources other than the maternal blood stream, e.g. uterine glands and the like.

Holoblastic. *See* Cleavage.

Homoiogenetic induction. The power acquired by a part of an embryo, at the

noiogenetic induction (*cont.*)
me time as it is determined for a certain
fferentiation, to induce another of the
me kind. Thus at the same time as the
eural plate is induced by the underlying
esoderm, it acquires the power of in-
ucing another neural plate if it is removed
d implanted into the blastocoele cavity
another embryo.

moioplastic transplantation. Transplan-
tion between individuals of the same
ecies.

moiotopic transplantation. Transplanta-
on between corresponding (identical) sites.

mologous. Said of organs or structures
hich have the same evolutionary origin
ough their functions may be very different.
ee Analogous.

ridisation. The fertilisation of an egg of
ne species by the sperm of another.

plantation. The addition of tissue, grafts,
emical fractions in an implant medium,
tc. to an embryo, without the removal of
nything from it.

ompatibility. Opposite of Affinity (q.v.),
= Roux's Cytochorism.

irect induction. The induction of a new
eural axis in competent ventral ectoderm by
chemical compound, not acting in the same
ay as the naturally occurring evocator, but
y liberating the masked evocator in the
eacting tissue.

ividuation. That part of the morpho-
enetic effect of the primary organiser in
eural induction, especially the regional
ifferentiation, which cannot with certainty
e referred back to the action of a single
hemical substance, the *Evocator*. Since it
eems probable that forces of field type are
volved in the regional differentiation of the
oked axis, the *Individual field* is spoken of.
he history of the word has already been
scussed (p. 125). It has, however, another
nbryological use which was not appre-
ated when it was introduced into causal
orphology. Neurologists have spoken of
e differentiation of local reflex systems,
haviour patterns, and the like, out of the
tal undifferentiated diffuse response of the
rly embryo to stimuli, as "individuation"
oghill[3]). This is not perhaps a very good
se, since it applies to *parts* of the organism,
ence not to true *individuals*. The word
emancipation" (q.v.) would be preferable.

uction. The morphogenetic effect brought
bout by an organiser, inductor, evocator,
tc. acting on competent tissue. *See also*
omoiogenetic, Direct, Indirect, Autono-
nous, Complementary, Palisade.

uctor. A piece of living tissue, dead
ssue, a chemical fraction, or a chemical
ubstance of known constitution, which
arries out an *induction* similar to that performed by an *organiser*. The word can thus be used indiscriminately for a number of types of inducing agent. To the primary and secondary organisers correspond primary and secondary inductors. (N.B. the term "secondary" here must be sharply distinguished from its use for the induced member of a twin, e.g. the "secondary" embryo as opposed to the "host" embryo.) *See also* Nuclear inductor.

Infection (*Weckung*). The appearance of inductive power in a piece of tissue not originally possessing it, when that tissue has been placed for some time in the normal organiser centre.

Ingression. An inward movement of cells in the amphibian blastula, especially the yolk endoderm. (In coelenterate embryology the word has another meaning.)

Intermediate. *See* Mosaic.

Intersex. *See* Gynandromorph.

Localisation (*Sonderung*). The separation of phases ("plasms") in mosaic eggs, distinguishable cytologically, and each associated with a specific subsequent differentiation. Such phases or regions are often characterised by pigments or granules which led to the old term "organ-forming substances". Since the cytoplasm of these regions, rather than the visible elements within them, must be regarded as responsible for the morphogenetic specificity, and since evocators now deserve rather to be called "morphogenetic substances", the old term should be abandoned.

Macromere. *See* Blastomere.

Material determination. Determination of a part of an embryo to perform a certain type of histogenesis or morphogenesis; as opposed to Dynamic determination (q.v.).

Maturation. The sum total of processes whereby the egg is prepared for fertilisation, including oogenesis, the growth of the ovum, and the final nuclear changes.

Merisis. Growth in size due to cell-division.

Meroblastic. *See* Cleavage.

Merogony. The development of fragments of eggs. A fragment of an egg, containing the nucleus, may be fertilised by sperm (*Diploid merogony*). A fragment of an egg, not containing the nucleus, may be fertilised by sperm (*Andro-merogony*). A fragment of an egg, containing the nucleus, may be removed from the remainder, after fertilisation by sperm but before nuclear fusion (*Gyno-merogony*), or the same experiment may be made using artificial fertilisation (*Parthenogenetic gyno-merogony*). Lastly, a fragment of an egg, not containing the egg-nucleus, can be fertilised by artificial means (*Parthenogenetic merogony*).

Mesoderm. The middle of the three cell- or germ-layers of classical embryology.

Metabolism. The sum total of chemical changes in the organism, including all catabolic and all synthetic processes, respiration, fermentation, etc.

Metathetely. The appearance of structures normally associated with an earlier stage of development, at a later stage of development, e.g. the retention of larval organs by insect pupae.

Metamorphosis. The sum total of changes, often very profound, by which the larval organism is transformed into the adult form (as tadpole into frog, caterpillar into butterfly, or pluteus into sea-urchin).

Metastasis. Spontaneous dispersion of a tumour from its original site in the body to other sites.

Micromere. *See* Blastomere.

Modulation. The reversible histological differentiations which occur as the final stages of the development of a tissue.

Morphogenesis. The coming-into-being of characteristic and specific form in living organisms.

Morphogenetic stimulus. A stimulus exerted by one part of the developing embryo upon another, leading to morphogenesis in the part reacting.

Morphallaxis (*Reparation*). Morphological modification or Regeneration without the formation of any new cells (opp. of true *Regeneration* or *Epimorphosis*).

Morphogenetic movements (*Gestaltungsbewegungen*). The modelling movements in differentiation which change the shape of tissues, e.g. the cell streams, whereby, as in gastrulation, the eventual positions of embryonic cells are attained. They include invagination, expansion, longitudinal extension, and dorsal convergence.

Mosaic. (1) as opposed to *Regulative.* In mosaic development the fates of all the parts of the embryo are already fixed at or before fertilisation, so that any localised ablation or injury will later be manifested as a complete lack of the part in question. In regulative development, however, injuries can be repaired, deficiencies made up, and indifferent added tissue organised into the pattern of the body. Mosaic eggs cannot form giant embryos by egg fusion nor dwarf ones by blastomere separation, and, generally speaking, twinning is much more restricted in them.

(2) in Genetics. Genetic mosaics are individuals the tissues of which contain cells of more than one genetic constitution. They may be due to somatic mutation, somatic crossing-over, or chromosome elimination.

(3) as opposed to *Intermediate*. Organisms vary not only as to the presence or absence of organs, but also to the extent to which any one organ is formed. An organism might have all its organs complete save one, but that one entirely absent; conversely, it might have them all, but imperfectly veloped. The former are, in this ser mosaics; the latter intermediates. T sense of the word is used mainly by work on Aphids.

Nachbarschaft (*Contiguity effects*). The m phogenetic effects produced by neighbou ing tissues or structures on a develop embryonic organ.

Neighbourwise (*Ortsgemäss*). Behaving aft transplantation in accordance with the ne situation in which the graft finds itself, i. plastic, pluripotent, undetermined. "Fal ing into step" with the new neighbou cells and going along with them to the morphogenetic destination.

Neoplasm, Neoplastic. A tumour, tumorous

Neoteny. Sexual maturity occurring in th larval period.

Neurogen. A term which may be used for th primary evocator and all other chemica substances which bring about neural indu tion in vertebrates (cf. *Carcinogen*, *Oestrogen*, etc.). Not to be confused with adjectives for muscular contractions, said to be neurogenic or aneurogenic (myogenic) according as t whether they require nervous stimulus or not.

Neurula. The stage in development at which the neural axis is fully formed and histogenesis rapidly proceeding.

Neutral medium. A situation for the culture of young undetermined parts of embryos such that they are not exposed to the action of unknown inductors (e.g. Holtfreter salt solution, as against chorio-allantoic grafting, yolk-sac implantations, or culture in eye- or body-cavity).

Nidicolous. Birds which hatch in a relatively undeveloped stage and hence have to remain in the nest highly dependent on their parents for some considerable time.

Nidifugous. Birds which hatch in a relatively highly developed stage, and can therefore leave the nest at once or almost so.

Nuclear inductor. A morphogenetic stimulating substance given off by the nuclear material bearing the factors of heredity (genes and chromosomes), usually operating only within the cell in question, but in some cases more diffusible.

Oestrogen. A chemical substance with activity analogous to that of the female sex hormones in the oestrous cycle.

Organiser. A living part of an embryo which exerts a morphogenetic stimulus upon another part or parts, bringing about their determination and the following histological and morphological differentiation. The organiser which acts first in development is known as the *Primary* or *First-Grade* Organiser; those acting at successive stages are known as *Secondary* (*Second-grade*) and *Tertiary* (*Third-grade*) Organisers. The appearance of the latter is generally de-

iser (*cont.*)
dent upon the prior function of the nary organiser, which therefore *seems* to nore manifold in its inductive effects. The mical substance emitted by an organiser alled an *Evocator*. *See also* Inductor.

ogenesis. The development of mor- logical differentiation.

topic transplantation. Transplantation ween corresponding (identical) sites in n a way that the graft maintains its mal orientation.

emäss. *See* Neighbourwise.

arity. The bringing forth of young from s developing outside the maternal body.

iviparity. The bringing forth of young n eggs developing within the maternal y, but having little or no connection h the maternal tissues.

de induction. A neural induction in ch the stimulus has not been sufficiently ng, or the mechanical conditions not ficiently favourable, to allow of the forma- of a well-shaped neural tube. Instead this, the histologically differentiated ral cells are arranged in palisade fashion und the implant.

iosis. The grafting together of two or re embryos, usually side by side, in order study their later mutual morphogenetic l endocrine effects.

enogenesis. The stimulation to de- pment, more or less complete, of an egg artificial means.

trance. The percentage of animals carry- a gene which manifest its effects. *See* Expressivity.

ocopy. The imitation of a morphological physiological character due to one geno- in an organism possessing a different type, by the action of external physical chemical factors, not leading to any ritable change.

critical period. A period in the de- pment of an organism during which effect produced by a gene can most lily be influenced or initiated by extern- applied factors.

type. The sum total of morphological physiological characters produced in an nism by the influence of its genotype.

nta. The organ formed at the junction ween foetal and maternal tissues and od streams. Its various types have been ssified morphologically and physiologic- (see p. 80).

n. A region of cytoplasmic localisation in saic eggs which gives rise later to a nite organ or organ system.

atic localisation. *See* Localisation.

city. Equivalent to *Pluripotence*; the diness of a part of the embryo for a e number of possible fates.

tropism. The causation of multiple cts by one genetic factor, probably not ctly, but because of an influence on metabolism which manifests itself in many different ways.

Pluripotent. In a condition of having a large number of possible fates; undetermined.

Polyploid. Containing integral multiples of the haploid number of chromosomes.

Polyploidogen. A chemical substance which, by inhibiting certain phases of nuclear division, gives rise to polyploid cells and tissues.

Potency. The use of this word should carry the undertone of "possibility", not "power" (in correct accord with its Aristotelian origin). Potency differs from competence in that it characterises the whole period of development, including the time before any competences have arisen. It makes no reference to any causal process. The expressions "self-differentiating potency" and "inductive potency" should therefore be avoided unless it is made clear that these qualities have not yet appeared, but may do so later. *See* Prospective Potency.

Presumptive. Having regard to the normal fate of the part in question, e.g. presumptive skin or presumptive ear-vesicle.

Prospective potency (*prospective Potenz*; *Potentialité totale*). The sum total of possible fates of any part of the embryo at the beginning of development. In regulative eggs prospective potency is much wider than prospective significance; in mosaic eggs the two are identical.

Prospective significance (*prospektive Bedeutung*; *Potentialité réelle*). The actual normal fate of any part of the embryo at the beginning of development.

Prothetely. The appearance of structures normally associated with a later stage of development, at an earlier stage of development, e.g. pupal organs in larval insects.

Recapitulation. The appearance, during the development of a higher organism, of structures or functions characteristic of developmental stages of lower organisms.

Recuperation. The reappearance of competence at late stages of development, as in e.g. the regenerative power of the adult ascidian after its mosaic embryonic development, or the lens competence believed to exist in the regenerating amphibian limb blastema.

Regeneration. The formation of part or whole of a new organ in place of one that has been removed. *See also* Wolffian regeneration.

Regional differentiation. The appearance of regional differences within the individuation field, e.g. the differentiation of an embryonic axis into cephalic and caudal ends, with all that that implies; or within organ districts such as that of the limb.

Regulation. The power of continuing normal or approximately normal development or regeneration in spite of experimental interference by ablation, addition (implantation),

Regulation (*cont.*)
exchange (transplantation), fusion, etc. The processes thus revealed operate in all embryos to a greater or lesser extent and may be regarded as part of the sum total of processes whereby the organism is rendered more or less independent of its environment. *See also* Fertilisation.

Regulative. *See* Mosaic.

Reintegration. The restoration to the organism of control by its individuation field, through the action of hormonal and neural factors during the period of functional differentiation, after the period of self-differentiation.

Reunition. The reconstitution of an organism after it has been separated into its component cells, not all types of which may be available, as in sponges.

Second-grade. *See* Organiser.

Secondary. This adjective has two senses; sometimes it is used as the equivalent of "second-grade", of organisers, but also for the embryo produced by an implanted organiser, as opposed to the host, or primary, embryo.

Self-differentiation (*Différentiation spontanée*). The differentiation of a tissue solely as the result of factors existing within itself, i.e. differentiation after determination has been accomplished (*see* Dependent differentiation). Any differentiation in explanted tissue is self-differentiation (*see* Bedeutungsfremde Selbstdifferenzierung).

Selfwise (*Herkunftsgemäss*). Behaving after transplantation in accordance with the old situation in which the graft had previously been, i.e. in accordance with its prospective significance, i.e. determined, self-differentiating, and no longer pluripotent. Refusing to "fall into step" with the new neighbour cells and go along with them to their morphogenetic destination.

Spaltung. A twin embryo in which the two neural axes are united posteriorly; it is due to a splitting of the morphogenetic movements into two streams, and may simulate an induction.

Stepwise inhibition. The successive inhibition of different organic processes by successively stronger applications of an externally acting agent.

Substrate. The substance acted on by an enzyme.

Tachyauxesis. *See* Heterauxesis.

Telolecithal. Having yolk accumulated at one point of the egg.

Teras. A monster caused by developmental deviation.

Teratology. The study of terata and their origin.

Teratoma. A more or less malignant assembly of tissues, well differentiated histologically, but showing varying degrees of morph logical differentiation, and embedded in t body of an otherwise normal fully develope organism. Some pathologists distinguis between the histologically differentiate type (*Embryoma*) and that showing bot histological and morphological differenti tion (*Teratoma* proper).

Thermophene. A curve relating quant tatively the effects of a gene to the tem perature at which development has take place.

Transplantation. The transfer of a piece tissue from one embryo to another; if th transfer is reciprocal between two embryos we speak of *Exchange* (*Austausch*). *See als* Autoplastic, Homoioplastic, Heteroplastic Xenoplastic, Homoiotopic, Heterotopic Orthotopic.

Trophallaxis. The exchange of secretions etc between individual members of a community of social insects, whereby social cohesion and caste differentiation are thought to b effected.

Trophic. An action of the nervous system in the absence of which muscle tonus fails and regeneration is impossible.

Trophoblast. The non-embryonic part of the mammalian blastocyst, which effects its implantation into the uterine wall, and part of which later develops into the foetal portion of the placenta.

Umhüllung. The wrapping of an inductor in sheets of competent ectoderm, in order to test inductive power.

Vegetal pole. That pole of the egg which contains most yolk and least cytoplasm.

Vegetalisation (*Endodermisation*). The shifting of the presumptive fate of tissue which should have been ectodermal, in echinoderm development.

Verlagerung. Shuffling of the blastomeres of an egg.

Verschmelzung. Fusion of two eggs together.

Vitelline membrane. The non-cellular membrane surrounding the yolk before development in the hen's egg, eventually superseded by the yolk-sac.

Viviparity. The maintenance of embryos during their development within the maternal body in relatively close contact with the maternal tissues or blood stream.

Wolffian regeneration. The regeneration of a new lens in the eye after the removal of the former one, from the upper margin of the iris.

Xenoplastic transplantation. Transplantation between individuals of different genera or widely distant species.

Yolk-sac. The extension of the blastoderm into a membrane surrounding the yolk in amniotes, specialised for absorption of nutritive materials.

TERMS AND CONCEPTS THE USE OF WHICH IS NOT RECOMMENDED

	See discussion on p.
Auxano-differentiation	506
Blastogen or **Blastomogen** (for Carcinogen)	—
Closed term	112
Determinant	101
Differential dichotomy	112
Double assurance (*doppelte Sicherung*)	157, 294, 308
Entelechy	119
Harmonious equipotential system	121, 140, 477
Heterogonic growth (for Heterauxetic)	533
Histo-differentiation	506
Isotropic (for Pluripotent)	112
Labile determination	112, 157
Mitogenetic rays	422
Nullipotent	436
Open term	112
Organ-forming substances	131
Segregation	112
Self-origination	112
Specific potency	112
Substrate (in a morphological sense, for Substratum)	153
Totipotent	121

Bibliography

"Parthians and Medes and Elamites, and the dwellers in Mesopotamia, in Judaea and Cappadocia, in Pontus and Asia, in Phrygia and Pamphylia, in Egypt and the parts of Libya about Cyrene, and sojourners from Rome, both Jews and proselytes, Cretans and Arabians, we do hear them speaking in our tongues the mighty works of God." Acts of the Apostles ii. 9–11.

NOTES

(1) In order to save space, the names of the journals are printed in abbreviated form. A list
these arbitrary abbreviations is given below.

(2) The difficulties caused by haphazard spelling of Slavonic names are overcome here by
adoption of standard forms, as follows:

Russian endings: -vitch, -vsky, -ev, -ov.
Polish endings: -wicz, -wski.
Czech and Yugo-slav endings: -vić, -vać.
Bulgarian endings: -eff, -off.

(3) Chinese names are romanised as far as possible according to Wade's system, and the hsing
family name precedes the hyphenated ming-dze.

KEY

TO ABBREVIATIONS USED IN THE BIBLIOGRAPHY

	Analyst
	Anat. Anzeiger
	Annals of Applied Biol.
	Atti d. R. Accad. dei Fisiocrit. d. Siena
	Archives d'Anat., d'Histol. & d'Embryol.
	Arbeiten a. d. Anat. Instit. d. k. Univ. Sendai
	Atti d. R. Accad. (Naz.) dei Lincei
	Archives d'Anat. Mic.
	Archiv f. Anat. u. Physiol. (Meckel's, later Müller's, later du Bois Reymond's)
	Annales d'Anat. pathol.
	Annales Acad. Sci. Fenn. A
	Atti d. R. Accad. d. Sci. Torino
SR	Archiv Anat., Histol. & Embryol. (U.S.S.R.) (formerly Russkiy Archiv. . .)
	Acta Biotheoretica
	Acta Biol. Exp. (Warszawa)
	Acta Biol. Latvica (formerly Bull. Soc. Biol. Latvia)
	Acta Brevia Nederland. de Physiol. Pharmacol. & Microbiol.
MNB	Annales & Bull. Soc. Roy. Sci. Med. & Nat. Bruxelles
SSR	Archiv Biol. Nauk. (U.S.S.R.) (or Archives des Sci. Biol.)
	Acta Cancrologica (Budapest)
	Annals Czechoslovak Acad. Agric. (Sbornik Ceskoslovenske Akad. Zemedelske)
	Archives de Biol.
	Annalen d. Chem. (Liebig's)
	Archives of Dermatol. & Syphilol.
	Archiv f. exp. Pathol. u. Pharmacol. (Schmiedeberg's)
	Ann. Entomol. Soc. Amer.
	Archiv f. exp. Zellforsch. (Erdmann's)
	Archivio di Fisiol.
	Ann. Farmacol. & Bioquim. (Buenos Aires)
	Archiv f. Dermatol. u. Syphilol.
	Archiv f. Entwicklungsmechanik (Roux's)
	Archiv f. Geflügelkunde
	Archiv f. Ophthalmol.
	Archivio di Farmacol. sper.
	Arkiv för Zool. (Upsala)
	Archiv f. Gynaekol.
	Angewandte Chem.
T	Archiv f. d. Geschichte d. Math., Naturwiss. u. Technik
R	Acta Gynaecol. U.S.S.R.
	Abhandl. d. kgl. Gesell. d. Wiss. zu Göttingen
	Anat. Hefte
	Archiv f. Hydrobiol. u. Planktonkunde
	Archivio Ital. di Anat. & Embriol.

AIB	Archives Ital. de Biol.
AIM	Annals of Internal Medicine
AIOP	Ann. de l'Instit. Océanogr. Paris
AIP	Archives internat. de Physiol. (Liège)
AIPS	Ann. de l'Institut Pasteur
AIPT	Archives internat. de Pharmacodynamie & Thérapie
AIUC	Acta of the Internat. Union against Cancer
AJA	Amer. Journ. Anat.
AJB	Amer. Journ. Bot.
AJBMS	Australian Journ. Exp. Biol. & Med. Sci.
AJC	Amer. Journ. Cancer
AJDC	Amer. Journ. Dis. Children
AJMS	Amer. Journ. Med. Sci.
AJOG	Amer. Journ. Obstet. & Gynaecol.
AJO	Amer. Journ. Ophthalmol.
AJOH	Amer. Journ. Hyg.
AJOP	Amer. Journ. Physiol.
AJOPA	Amer. Journ. Pathol.
AJOPS	Amer. Journ. Psychol.
AJPA	Amer. Journ. Physical Anthropol.
AK	Allattani Közlemenyek (Budapest)
AKC	Archiv. f. klin. Chirurg.
AKS	Archiv d. J. Klaus Stiftung f. Vererbungsforsch., Sozialanthropol. u. Rassenhyg. (Zürich)
ALRFS	Annali Lab. Ric. Fermenti Spallanzani
AMA	Archiv f. mik. Anat.
AMAD	Annales des maladies des Appareils Digestifs
AMBUSSR	Advances in Modern Biology (U.S.S.R.)
AMGE	Archives de Morphol. gén. & exp.
AMN	Amer. Midland Naturalist
AMNH	Annals & Mag. Nat. Hist.
AMS	Acta Med. Scand.
AMUO	Arbeiten a. d. med. Univ. Okayama
AN	Amer. Naturalist
ANM	Acta Nederland. Morphol.
ANP	Archives Néerland. de Physiol.
ANVH	Abhandl. (a. d. Gebiete d. Naturwiss. hrsg. vom) Naturwiss. Verein in Hamburg
ANYAS	Ann. New York Acad. Sci.
ANZJS	Austral. & New Zealand Journ. Surgery
AO	Annali di Ostet. & Ginecol.
AOB	Annals of Bot.
AOG	Archivio di Ostet. & Ginecol.
AOGS	Acta Obstet. & Gynaecol. Scand.
AOP	Archives of Pathol.
AP	Archiv f. d. g. Physiol. (Pflüger's)
APANL	Atti d. Pontif. Accad. Sci. Nuovi Lincei
APB	Archives de Physique Biol.
APBPC	Annales de Physiol. & Physico-chimie Biol.

APD	Acta Pediatrica
APPA	Archiv f. path. Anat. u. Physiol. (Virchow's)
AR	Anat. Record
ARB	Annual Rev. Biochem.
ARBA	Annual Reps. Brit. Assoc. Advancement of Sci.
ARCS	Annual Reps. Chem. Soc.
ARFA	Rapp. de l'Assoc. Français pour l'Avancement des Sci.
ARP	Annual Rev. Physiol.
ART	Amer. Rev. Tuberculosis
ASB	Archivio di Sci. Biol.
ASBA	Annales de la Station Biol. d'Arcachon
ASBSP	Annuario d. Staz. Bacol. Sper. Padova
ASFFF	Acta Soc. pro Fauna & Flora Fennica
ASNZ	Annales des Sci. Nat. (Zool.)
ASOJ	Acta Soc. Ophthalmol. Jap.
ASRZB	Annales de la Soc. Roy. Zool. & Malacol. Belg.
AT	Arbeiten. d. Ungar. Biol. Forschungsinstit. Tihany
ATT	Archiv f. Tierernährung u. Tierzucht
AZ	Acta Zoologica (Stockholm)
AZEG	Archives de Zool. exp. & gén.
AZF	Archiv f. Zellforsch.
AZI	Archivio Zool. Ital.
AZJ	Annotationes Zool. Jap.
AZSZBFV	Ann. Zool. Soc. Zool. Bot. Fenn. Vanamo
B	Biomorphosis
BAA	Bull. de l'Assoc. des Anat.
BAEC	Bull. de l'Assoc. pour l'Étude de Cancer
BAM	Bull. de l'Acad. de Méd. Paris
BAMNH	Bull. Amer. Mus. Nat. Hist.
BAMR	Boll. d. R. Accad. Med. Roma
BAPS	Bull. internat. Acad. Pol. Sci. & Lett. (Kraków)
BARBS	Bull. de l'Acad. Roy. Belg.
BARMB	Bull. de l'Acad. Roy. de Méd. Belg.
BASUSSR	Bull. Acad. Sci. U.S.S.R.
BB	Biol. Bull.
BBFB	Bull. Biol. Fr. & Belg. (formerly Bull. Sci. Fr. & Belg.)
BBMEUSSR	Bull. Biol. & Med. Exp. U.S.S.R.
BC	Biol. Centralbl.
BCC	Biochem. Centralbl.
BDBG	Ber. d. deutsch. Bot. Gesell.
BDCG	Ber. d. deutsch. Chem. Gesell.
BDJ	Brit. Dental Journal
BDZ	Boll. di Zool.
BER	Bull. Entomol. Res.
BFASUK	Bull. Fac. Sci. Agric. Univ. Kyushu
BG	Biol. Generalis (Wien)
BGA	Bibliographia Genetica ('s Graavenhage)
BHA	Bull. de l'Histol. appl.
BIZUR	Boll. Instit. Zool. Univ. Roma
BJ	Biochem. Journ.
BJEP	Brit. Journ. Exp. Pathol.
BJHH	Bull. Johns Hopkins Hospital (Baltimore)
BJR	Brit. Journ. Radiol.

BJS	Brit. Journ. Surgery
BL	Biol. Listy (Prag)
BLL	Bratislavske Lekarsky Listy
BLZAM	Boll. Lab. Zool. Agrar. Milano
BM	Biometrika
BMA	Bergens Mus. Aarbog
BMDIMBL	Bull. Mt. Desert Island Ma Biol. Lab.
BME	Bruxelles Méd.
BMHN	Bull. Mus. d'Hist. Nat. Paris
BMJ	Brit. Med. Journ.
BMKDVS	Biol. Medd. Kgl. Dansk Videns Selskab
BMLZACUG	Boll. Mus. & Lab. Zool. & A Comp. Univ. Genova
BN	Brain
BNYAM	Bull. New York Acad. Med.
BOG	Bot. Gazette
BOGG	Ber. d. Oberhess. Gesell. f. Natur Heilk. zu Giessen.
BOR	Bot. Rev.
BOZ	Bot. Zeitung
BOZJ	Bot. & Zool. Jap.
BP	Bull. Math. Biophysics
BPAAP	Beitr. z. pathol. Anat. u. allg. Path (Ziegler's)
BR	Biol. Rev.
BSAP	Bull. Soc. Anthropol. Paris
BSBG	Bull. Soc. Belg. Gynaecol.
BSCB	Bull. Soc. Chimie Biol.
BSCF	Bull. Soc. Chimie de Fr.
BSHA	Bull. Soc. Hyg. alimentaire
BSIBS	Boll. Soc. Ital. Biol. Sper.
BSNM	Bull. Soc. Naturalistes Moscow
BSNN	Boll. Soc. Naturalisti Napoli
BSPP	Bull. Soc. Philomath. Paris
BSRNPPE	Bull. Soc. Roumaine de Neur Psychiatr., Psychol. & Endocrin
BSSAR	Bull. Sect. Sci. Acad. Roumaine
BSZF	Bull. Soc. Zool. de Fr.
BTBC	Bull. Torrey Bot. Club
BTS	Biochim. & Terap. Sper.
BUSSR	Biochemia (U.S.S.R.)
BZ	Biochem. Zeitschr.
BZUSSR	Biol. Zhurn. U.S.S.R.
C	Cytologia (Tokyo)
CA	Chem. Abstr.
CAC	Centralbl. f. agric. Chem. u. Landw. (Biedermann's)
CAP	Centralbl. f. allg. Pathol.
CAR	Cancer Rev.
CB	Centralbl. f. Bakteriol. u. Parasit kunde
CBSSF	Commentat. Biol. Soc. Sci. Fe (Helsinki)
CBTI	Contrib. Boyce-Thompson Instit.
CCE	Carnegie Contrib. to Embryol.
CCP	Colorado College Pub.
CDE	Chemie. d. Erde
CG	Centralbl. f. Gynaekol.
CI	Chem. & Industry (Journ. Soc. Che Industry)
CIWP	Carnegie Instit. of Washington P lications

	Carnegie Instit. of Washington Yearbook
	Chinese Journ. Exp. Biol.
	Chinese Journ. Physiol.
	Canadian Journ. Res.
	China Med. Journ.
	Centralbl. f. med. Wiss.
	Collecting Net (Woods Hole)
	Clinica Ostet.
	Chem. Products
	Chem. Rev.
	Comptes Rend. Assoc. Anat.
	Comptes Rend. hebdomadaires Acad. Sci. Paris
SSR	Comptes Rend. Acad. Sci. U.S.S.R.
	Comptes Rend. Soc. Biol.
	Comptes Rend. Soc. Sci. Varsovie (Warszawa)
	Comptes Rend. Trav. Lab. Carlsberg
	Current Sci. (Bangalore)
B	Cold Spring Harbor Symposia on quantitative Biology
	Colloid Symposium Monogr.
	Centralbl. f. Tierernährung
	Cornell Veterinarian
	Chem. Weekblad
	Discovery
	Deutsches Archiv f. klin. Med.
	Der Biologe
	Deutsche Med. Wochschr.
	Ecology
	Ergebn. d. Anat. u. Entwicklungsgesch.
	Ergebn. d. allg. Pathol. u. pathol. Anat.
	Ergebn. d. Biol.
	Ergebn. d. Enzymforsch.
	Ergebn. d. inn. Med. u. Kinderheilkunde
	Erkenntnis
	Ecological Monographs
	Edinburgh Med. Journ.
	Encéphale (Journ. mensuel de Neurol. & Psychiatr.)
	Endokrinologie (Leipzig)
	Endocrinology (Glendale, Calif.)
	Ergebn. d. Physiol.
	Ergebn. d. Vitamin- u. Hormon-Forsch.
	Exp. Sta. Record
	Enzymologia
	Folia Anat. Jap.
	Forschungen u. Fortschritte
	Fermentforsch.
	Folia Gynaecol.
	Forschungen a. d. Geb. d. Röntgenstrahl.
	Folia Haematol.
	Finska Läkaresällskapets Handlingar
	Folia Morphol. Hispanica (Valencia)
	Folia Morphol. Polonica (Warszawa)
	Folia Pharmacol. Jap.
FZ	Fortschritte d. Zool.
FZP	Frankfurter Zeitschr. f. Pathol.
G	Growth
GA	Genetica ('s Graavenhage)
GC	Ginecologia (Firenze)
GHR	Guy's Hospital Reps. (London)
GO	Gynécologie & Obstétrique
GR	Genesis (Roma)
GS	Genetics (Princeton)
H	Hilgardia (Journ. Agric. Sci. Calif. Agric. Exp. Sta.)
HB	Human Biol.
HCA	Helvetica Chem. Acta
HJ	Hibbert Journ.
IEC	Industrial & Eng. Chem. (formerly Journ. Industr. & Eng. Chem.)
IJMR	Indian Journ. Med. Res.
IRGHH	Internat. Rev. d. ges. Hydrobiol. u. Hydrogr.
IRJMS	Irish Journ. Med. Sci.
ISCJS	Iowa State Coll. Journ. Sci.
IZPCB	Internat. Zeitschr. f. physik.-chem. Biol. (dead)
JA	Journ. Anat. (continuation of Journ. Anat. & Physiol.)
JACS	Journ. Amer. Chem. Soc.
JACSJ	Journ. Agric. Chem. Soc. Jap.
JAMA	Journ. Amer. Med. Assoc.
JAOAC	Journ. Assoc. Official Agric. Chemists
JAP	Journ. Anat. & Physiol.
JAPA	Journ. Amer. Pharmacol. Assoc.
JAPP	Journ. Applied Physics (formerly Physics)
JAR	Journ. Agric. Res.
JAS	Journ. Agric. Sci.
JAVA	Journ. Amer. Vet. Assoc.
JBC	Journ. Biol. Chem.
JCCP	Journ. Cellular & Comp. Physiol.
JCE	Journ. Chem. Educ.
JCI	Journ. Clin. Investigation
JCMA	Journ. Chosen Med. Assoc.
JCN	Journ. Comp. Neurol.
JCP	Journ. de Chim. Physique
JCPS	Journ. Comp. Psychol.
JCR	Journ. Cancer Res.
JCS	Journ. Chem. Soc.
JCSJ	Journ. Chem. Soc. Jap.
JCST	Journ. Coll. Sci. Imp. Univ. Tokyo
JDAP	Journ. de l'Anat. & Physiol.
JDR	Journ. Dental Res.
JE	(Brit.) Journ. Endocrinol.
JEB	Journ. Exp. Biol. (formerly Brit. Journ. Exp. Biol.)
JECB	Journ. Econ. Biol.
JEE	Journ. Econ. Entomol.
JEM	Journ. Exp. Med.
JEZ	Journ. Exp. Zool.
JFI	Journ. Franklin Instit. Philadelphia
JFSHIU	Journ. Fac. Sci. Hokkaido Imp. Univ.
JFSUT	Journ. Fac. Sci. Imp. Univ. Tokyo
JG	Journ. Genetics

JGP	Journ. Gen. Physiol.
JGPS	Journ. Gen. Psychol.
JH	Journ. Heredity
JI	Journ. Immunol.
JID	Journ. Infectious Dis.
JJB	Journ. Biochem. (Tokyo)
JJCR	Gann; Jap. Journ. Cancer Res.
JJEM	Jap. Journ. Exp. Med.
JJG	Jap. Journ. Genetics
JJMSA	Jap. Journ. Med. Sci. (I, Anat.)
JJMSB	Jap. Journ. Med. Sci. (II, Biochem.)
JJMSP	Jap. Journ. Med. Sci. (III, Biophysics)
JJOG	Jap. Journ. Obstet. & Gynaecol.
JJZ	Jap. Journ. Zool.
JK	Jahrb. f. Kinderheilkunde
JLSZ	Journ. Linn. Soc. (Zool.)
JM	Journ. Morphol. (for a period was Journ. Morphol. & Physiol.)
JMBA	Journ. Marine Biol. Assoc. (Great Britain)
JMFUS	Jahrb. d. med. Fak. d. Univ. Sofia
JMM	Journ. Mammalol.
JMR	Journ. Med. Res.
JMS	Journ. Mental Sci.
JMSHNY	Journ. Mt Sinai Hosp. New York
JN	Journ. Nutrition
JNCI	Journ. Nat. Cancer Instit. (U.S.)
JNP	Journ. Neurophysiol.
JNPP	Journ. Neurol. & Psychopathol.
JNYES	Journ. New York Entomol. Soc.
JO	Journ. f. Ornithol.
JOGBE	Journ. Obstet. & Gynaecol. of the Brit. Empire
JOH	Journ. Hyg.
JOP	Journ. Physiol.
JP	Journ. Pediatr.
JPA	Journ. Parasitol.
JPB	Journ. Pathol. & Bacteriol.
JPC	Journ. Physical Chem.
JPE	Journ. de Psychol.
JPET	Journ. Pharmacol. & exp. Therapeutics
JPPG	Journ. de Physiol. et de Pathol. Gén.
JPS	Journ. Philos. Studies
JRMS	Journ. Roy. Mic. Soc.
JSHU	Journ. Sci. Hiroshima Univ.
JSI	Journ. Sci. Instruments
JSM	Journ. State Med.
JUGP	Jahresber. ü. d. ges. Physiol. u. exp. Pharmacol.
JWBO	Jahrb. f. wiss. Bot.
JZN	Jenaische Zeitschr. f. Naturwiss.
K	Kosmos (Journ. Soc. Pol. Nat. Kopernik)
KB	Kolloid Beihefte (formerly Kolloid Chem. Beihefte)
KIZ	Kyoto Igakkwai Zasshi (Kyoto Med. Journ.)
KJM	Keijo Journ. Med.
KMA	Klin. Monatsbl. f. Augenheilkunde
KNVSF	Kgl. Norske Videnskab Selskabs Forhandlinger
KOKK	Közlemények az összehesonlitó élet-és Kórtan Köreböl

KW	Klin. Wochschr. (formerly B Klin. Wochschr.)
KZ	Kolloid Zeitschr.
L	Lancet
LC	La Cellule (Louvain)
LJB	Bull. Scripps Oceanograph. I La Jolla, Calif.
LT	Landbouwkundig Tidschr.
LUA	Lunds Univ. Årsskrift
MAPS	Mem. Acad. Pol. Sci.
MARBS	Mém. Acad. Roy. Belg. (Sci.)
MASIB	Mem. d. R. Accad. Sci. dell' Ist Bologna
MASU	Mem. Acad. Sci. Ukraine (U.S.S
MC	Monatshefte f. Chemie
MCSK	Mem. Coll. Sci. Kyoto Imp. Univ
MDS	Medycyna Doswiadczalna i Społe (Warszawa)
ME	Micro-entomology (Contrib. to tomol. from Stanford Univ.)
MGG	Monatschr. f. Geburtshilfe u. Gyn kol.
MINPERP	Mem. Instit. Nat. Pol. d'É Rurale de Puławy
MJ	Morphol. Jahrb. (Gegenbaur's)
MJA	Med. Journ. Australia
MJBG	Mitt. d. Jap. Biochem. Gesell.
MJGG	Mitt. d. Jap. Gesell. f. Gynaekol.
MK	Monatschr. f. Kinderheilkunde
MMAK	Mitt. a. d. med. Akad. Kyoto
MMFUT	Mitt. a. d. med. Fak. Univ. Toky
MMRHNB	Mémoires du Mus. Roy. d'H Nat. Belg.
MOG	Monit. Ostet. & Ginecol.
MRAI	Mem. d. R. Accad. Ital. (Roma)
MSPEUSSR	Materials for the Study of Pathol. of Emb. Devel. in B (U.S.S.R.)
MZI	Monit. Zool. Ital.
N	Nature
NAV	North American Veterinarian
NEJM	New England Journ. Med.
NGWG	Nachr. v. d. Gesell. d. Wiss. Göttingen
NIZ	Nagasaki Igakkwai Zasshi (Naga Med. Journ.)
NJMS	Nagoya Journ. Med. Sci.
NTG	Nederlandsch Tijdschrift v. Gen kunde
NTVKI	Nordisk Tidskr. för Vetenskap, Ko o Industrie
NW	Naturwissenschaften
O	Osiris
OIZ	Okayama Igakkwai Zasshi (Okaya Med. Journ.)
OJDI	Oriental Journ. Dis. Infants
OJS	Ohio Journ. Sci.
OJVSAI	Onderstepoort Journ. Vet. Sci. Anim. Industry
OMaly	Ber. ü. d. ges. Physiol. u. Pharma (Maly's)

	Orvoskepzes (Budapest)
A	Occasional Pub. Amer. Assoc. Adv. Sci.

	Protoplasma
	Parasitology
S	Proc. Amer. Acad. of Arts & Sciences
	Philippine Agric.
SSR	Probl. of Animal Husbandry (U.S.S.R.)
	Proc. Amer. Philos. Soc.
MED	Proc. Assoc. for Res. in Nervous & Mental Disease
	Proc. Aristot. Soc.
P	Proc. Amer. Soc. Animal Production
	Proc. Cambridge Antiquarian Soc.
	Proc. Cambridge Philos. Soc. (Biol.)
	Proc. Entomol. Soc. Philadelphia
SR	Probl. d. Ernährung (U.S.S.R.)
	Polska Gazeta Lekarska
	Proc. Indian Acad. Sci.
	Proc. Imp. Acad. Tokyo
SR	Probl. d. Inkubation (U.S.S.R.)
A	Proc. Kon. Akad. Wetenskap Amsterdam
SW	Proc. Linnean Soc. New South Wales
	La Presse Médicale
	Prensa Med. Argentina
W	Proc. Nat. Acad. Sci. (Washington)
	Peiping Nat. Hist. Bull.
S	Proc. Nova Scotia Instit. Sci.
	Phytopathology
	Plant Physiol.
	Proc. Physical Soc. (London)
	Physiol. Rev.
E	Proc. Roy. Physical Soc. Edinburgh
	Proc. Roy. Soc. (London)—before division into two parts
	Proc. Roy. Soc. A (London)
	Proc. Roy. Soc. B (London)
	Proc. Roy. Soc. Edinburgh
	Proc. Roy. Soc. Med. (London)
	Poultry Sci.
I	Proc. Soc. Exp. Biol. & Med.
	Psychol. Rev.
	Proc. Sci. Soc. Warsaw (Warszawa)
	Pubbl. d. Staz. Zool. Napoli (formerly Mitteilungen a. d.)
A	Philos. Trans. Roy. Soc. A. (London)
B	Philos. Trans. Roy. Soc. B. (London)
	Physiol. Zool.
SSR	Probl. d. Zootechn. & Exp. Endocrinol. (U.S.S.R.)
	Proc. Zool. Soc. (London)
SR	Physiol. Zhurn. (U.S.S.R.)

	Quart. Journ. Exp. Physiol.
S	Quart. Journ. Mic. Sci. (formerly Journ. Mic. Sci.)
	Quart. Journ. Pharmacy & Pharmacol.
	Quart. Rev. Biol.

	Redia (Giornale di Entomol.; Firenze)
	Radiology
	Rendiconti d. R. Accad. (Naz.) dei Lincei

RCT	Rassegna di Clin. Terap. e. Sci. affini
RDB	Riv. di Biol.
RG	Radiobiol. Gen.
RGS	Rev. gén. des Sci.
RIG	Riv. Ital. di Ginecol.
RLAUR	Ricerche d. Lab. Anat. Univ. Roma
RM	Ricerche d. Morfol. (Roma)
RPS	Riv. di Patol. sper.
RS	La Ricercha Sci.
RSAB	Rev. Soc. Argentina de Biol.
RSEIQ	Riv. Sudamer. Endocrinol., Immunol. & Quimoterap.
RSZ	Rev. Suisse de Zool.
RTBN	Recueil des Trav. Bot. Nederland
RTCPB	Recueil des Trav. Chim. des Pays-Bas
RUB	Rev. de l'Univ. de Bruxelles
RZR	Rev. Zool. Russe

S	Science (New York)
SA	Scientific Agriculture
SAIMRP	South African Instit. Med. Res. Pub.
SAJS	South African Journ. Sci.
SAWB	Sitzungsber. d. preuss. Akad. Wiss. Berlin
SAWH	Sitzungsber. d. Akad. Wiss. Heidelberg
SAWW	Sitzungsber. d. Akad. Wiss. in Wien
SC	Scientia
SGMPM	Sitzungsber. d. Gesell. f. Morphol. u. Physiol. in München
SGNFB	Sitzungsber. d. Gesell. Naturforsch. Freunde zu Berlin
SGO	Surgery, Gynaecol. & Obstet.
SGY	Surgery
SIDT	Stud. Instit. Div. Thom. (Cincinnati)
SIKMJ	Sei-i-Kwai Med. Journ.
SK	Suomen Kemistilehti (Acta Chem. Fennica)
SKAP	Skand. Archiv f. Physiol.
SKT	Svensk Kemisk Tidskrift
SM	Scientific Monthly
SMA	Semana Med. (Buenos Aires)
SMW	Schweiz. Med. Wochschr.
SNGB	Sitzungsber. d. naturforsch. Gesell. Bern
SNVAO	Skr. udg. av det Norske Videnskaps Akad. i Oslo
SP	Science Progress
SPHNGCR	Comptes Rend. Soc. Phys. Hist. Nat. Genève
SPHNGM	Mem. Soc. Phys. Hist. Nat. Geneva
SPMGW	Sitzungsber. d. phys.-med. Gesell. zu Würzburg
SRICRS	Sci. Reps. Imp. Cancer Res. Fund (London)
SRMRC	Spec. Reps. Med. Res. Council (Great Britain)
SRTIU	Sci. Reps. Tohoku Imp. Univ.
SSR	School Sci. Rev.

TAFS	Trans. Amer. Fisheries Soc.
TAMS	Trans. Amer. Mic. Soc.
TB	Tabulae Biol. (Period.)
TDDUSSR	Trans. on the Dynamics of Devel. (U.S.S.R.)

TES	Trans. Entomol. Soc. (London)
TFS	Trans. Faraday Soc.
TIEMUM	Trans. Instit. Exp. Morphogen. Univ. Moscow (U.S.S.R.)
TH	Tidskr. för Hermetikind.
TIN	Trav. de l'Instit. Nencki (Warszawa)
TIPNUSSR	Trudy Instit. Physiol. Narkompros (U.S.S.R.)
TIZBASU	Trav. de l'Instit. Zool. & Biol. Acad. Sci. Ukraine (U.S.S.R.)
TJEM	Tohoku Journ. Exp. Med.
TJPS	Trans. Jap. Pathol. Soc.
TLEBZM	Trans. Lab. Exp. Biol. Zoopark Moscow (U.S.S.R.)
TLZEMAM	Trav. du Lab. Zool. & Exp. Morphogén. Anim. Moscow (U.S.S.R.)
TNDV	Tijdschr. d. Nederlandsche Dierkund. Vereeniging (continued as Archives Néerland. de Zool.)
TNISI	Trans. Nat. Instit. Sci. India
TOSL	Trans. Obstet. Soc. (London)
TPRSA	Trans. & Proc. Roy. Soc. Australia
TRSC	Trans. Roy. Soc. Canada (Sect. V)
TSBE	Trans. Soc. Brit. Entomologists
TSBR	Trav. de la Stat. Biol. Roscoff
TSZW	Trav. de la Stat. Zool. Wimereux
TZS	Trans. Zool. Soc. (London)
U	Umschau (Frankfurt)
UBZ	Ukrainian Biochem. Zhurn. (later Biochem. Zhurn. Acad. Sci. Ukraine, U.S.S.R.)
UCM	Mem. Univ. Calif.
UCPP	Univ. Calif. Pub. in Physiol.
UCPZ	Univ. Calif. Pub. in Zool.
UL	Ugeskrift for Laeger (Danish Med. Journ.)
ULF	Upsala Läkareförenings Forhandlingar
UMN	Unterrichtsblätter f. Math. u. Naturwiss.
UMZ	Med. Zhurn. Acad. Sci. Ukraine, U.S.S.R.
UOB	Univ. Oklahoma Bull.
UPB	Univ. Pittsburgh Bull.
USBFIR	United States Fisheries Bureau Investigations Reports (formerly Bull. U.S. Fish Commission)
USNMP	United States National Mus. Proc.
USPHR	United States Public Health Reports
UW	Unsere Welt (Godesberg)
VDAG	Verhandl. d. deutsch. Anat. Gesell. (suppl. vols of Anat. Anzeiger)
VDPG	Verhandl. d. deutsch. Pathol. Gesell. (suppl. vols of Centralb. f. allg. Pathol.)
VDZG	Verhandl. d. deutsch. Zool. Gesell. (suppl. vols. of Zool. Anzeiger)
VIVTAL	Verhandl. d. Internat. Vereinigung f. theor. u. angew. Limnologie
VNGB	Verhandl. d. naturforsch. Gesel Basel
VNGZ	Vierteljahrschr. d. naturforsch. Ge in Zürich
VOUSSR	Vopros. Onkol. (U.S.S.R.)
VPMGW	Verhandl. d. phys.-med. Gesell. Würzburg
VRUSSR	Vestnik Röntgenol. & Radiol. (U.S.S.R.)
VSNFG	Verhandl. d. Schweiz. Naturfors Gesell.
W	Wszechswiat (Warszawa)
YJBM	Yale Journ. Biol. & Med.
YMaly	Ber. ü. d. wiss. Biol. (Maly's)
ZA	Zoologica (Sci. Contribs. New Yo Zool. Soc.)
ZAE	Zeitschr. f. Anat. u. Entwicklung geschichte
ZAP	Zeitschr. f. allg. Physiol. (dead)
ZAZ	Zool. Anzeiger
ZB	Zeitschr. f. Biol.
ZE	Zeitschr. f. angewandte Entomol.
ZEBUSSR	Zhurn. Exp. Biol. (U.S.S.R.)
ZF	Zeitschr. f. Fischerei u. d. Hilfswiss
ZGEM	Zeitschr. f. d. ges. exp. Med.
ZGG	Zeitschr. f. Geburtshilfe u. Gynäko
ZH	Zeitschr. f. Hyg.
ZIAV	Zeitschr. f. induktive Abstamm. Vererbungslehre
ZJAO	Zool. Jahrb. (Abt. f. Anat. Ontog.)
ZJS	Zool. Jahrb. (Abt. f. Systematik)
ZJZP	Zool. Jahrb. (Abt. f. allg. Zool. Physiol.)
ZKF	Zeitschr. f. Krebsforsch.
ZKH	Zeitschr. f. Kinderheilkunde
ZKL	Zeitschr. f. Konstitutionslehre
ZKM	Zeitschr. f. klin. Med.
ZMA	Zeitschr. f. Morphol. u. Anthropol.
ZMAF	Zeitschr. f. mik. Anat. Forsch. (Jahr f. Morphol. u. mik. Anat. Ab II)
ZMJ	Zool. Mag. Jap.
ZMOT	Zeitschr. f. Morphol. u. Ökol. d. Tie
ZP	Zoologica Poloniae
ZPC	Zeitschr. f. physiol. Chem. (Hopp Seyler's)
ZPCUSSR	Zhurn. Physik. Chem. U.S.S.R.
ZTZ	Zeitschr. f. Tierzüchtung u. Züc tungsbiol. (now Zeitschr. f. Züc tung, Abt. B; Tierzücht. u. Züc tungsbiol. einschl. Tierernährung
ZUNG	Zeitschr. f. Untersuch. Nahr. Genuss-Mittel (now Zeitschr. Untersuch. d. Lebensmittel)
ZVP	Zeitschr. f. vergl. Physiol.
ZWZ	Zeitschr. f. wiss. Zool.
ZZFMA	Zeitschr. f. Zellforsch. u. mik. Ana
ZZUSSR	Zool. Zhurn. U.S.S.R.

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INDEXES

By Margaret Miall

GENERAL INDEX

N.B. Figures in heavy type indicate the principal point of reference.

cetabularia, 417
cetonitrile, 454
cheiropodia, 376
chondroplasia, 385
cid production by echinoderm eggs, 572
cidosis, *see* *p*H
ctivation, 188, 355
ctivation centre, 460 ff.
denylpyrophosphate, 119, 612 ff.
drenal
  in elasmobranch reproductive cycle, 43
  in laying hen, 3
drenal cortex hormone, domain of specificity, 253, 262
drenalin, 609
ffinity effects, *see* Tissue
lbinism, 405
lga (*Acetabularia*), 417
lkali reserve, in fish eggs, 585
lkaloids, domain of specificity, 261, 262
llantoic liquid, 93, 606
lantois, 67 ff., 82 ff., 259
lelomorphs, 400 ff.
llometry, 533
llomorphosis, 533
mino-acids
  action on differentiation, 513
  excretion, 50 ff.
  in hen's egg, 620
mmocoete, 451
mmonia excretion, 50 ff., 625
mnion, **67** ff., 82, 146, 324, 616 ff.
mniotic liquid, 93, 315, 606
moebae, 419, 422, 425, 661, 671
mphibia
  determination, 103 ff.
  development, 141–320
  egg, carbohydrate metabolism, 617 ff.
  egg, catalase activity, 604
  egg, centrifugation, 658
  egg, constitution, 6, 25, 34, 524
  egg, nuclein metabolism, 634
  egg, osmotic pressure, 606
  egg, respiration, 587 ff., 605
  egg, respiratory enzymes, 604, 634
  egg, water supply, 34 ff.
Amphibia (*cont.*)
  egg, yolk utilisation, 73 ff.
  exogastrulation, 237
  eye-cup and lens induction, 225, 291
  eye development, 105
  fate-map, 321 ff.
  gastrulation, 674
  limb-bud differentiation, 667
  metamorphosis, 447
  mouth formation, induction of, 342
  ovoviviparity, evolution of, 47 ff.
  parental care, 32
  parthenogenesis, 352
  regeneration, 240, 430
  sex-hormones, 312 ff.
  temperature, influence on embryo, 337 ff.
  transplantations, 349
  ureotelic metabolism, 52 ff.
  vitamin C in embryo, 616
Anaemia, 22, 375, 378, 649 ff.
Anaerobiosis
  fish eggs, 585
  amphibian eggs, 588
  mammalian embryos, 598
Androgenesis, 357
Androgens, 88, 262 ff., 315
Andromerogons, 358
Androsterone, 252, 262, 317
Anidians, 223, 327
Animal and vegetal poles, electrical properties, 221
Animalisation, 477
Animals, comparisons between, 6, 34, 173, 228, 233, 279, 298, 321 ff., 334, 365 ff., 420 ff. 500, 602, 609, 620, 624, 641, 648
Annelids, 132, 140
Anophthalmia, 368
Anoxaemia, 648
Ant, 309
Anti-anaemia principle, 649
Antibodies, 30 ff.
Antigens, 30 ff., 349
Anti-rhachitic compounds, *see* Vitamin D
Anuran amphibia and cancer, 240
Aphids, 118
Arachnids, 50

Archenteron, 142, 152, 323, 500
Arginine, 52, 54, 620
Aristapedia, 378
Armadillo, Texan 9-banded, 228
Arthropods
  egg constitution, 27 ff.
  eye abnormalities, 397, 407
  nuclear division without cell division, 509
  respiration, 577
Ascidians, 131, 134, 325
  fate-map, 320 ff.
  ovoviviparity, 33
Ascorbic acid, *see* Vitamin C
Ash, 32 ff., 45, 560
Astacin, 210, 249, 652
Astaxanthin, *see* Astacin
Auxin, 109 ff., 185, 212 ff., 266
Avian egg
  albumen, 177
  antigens and antibodies, 30
  blastoderm, 223
  blastoderm, neoplasmoid, 258
  carbohydrate metabolism, 607
  cell-division and – SH, 426
  constitution, 6–24, 34
  determination, 330 ff.
  embryo, sex-hormones, 313
  embryo, wet and dry weights, 540
  maturation, 3 ff.
  metabolism, 48 ff., 427 ff.
  respiration, 591
  respiratory enzymes, 604
  species-specificity in transplantations, 350
  teratomata, 237
  twinning, 229 ff.
  uric acid formation, 55
  water conservation, 36
Avitaminosis, 23 ff., 264
Axial gradients, 97, 605
Axolotl, 33, 38, 74, 200, 342, 344, 364, 434, 436, 441, 448, 450, 666

Bacteria, 30 ff., 400, 512, 514
Barbiturates, 343
Basic ions in egg, 17
Bee, 46, 310
Beetle
  Black-, 56
  May-, 56
  Potato-, 50
Bile acids, 250, 262
Bile pigments, 647
Bios factors, 213
Birds
  blood factor in parthenogenesis, 355
  eggs, *see* Avian egg
  genes, 390
  growth, 540
  growth without differentiation, 507
  sex-reversal, 314
Birefringence, 8, 27, 661, 671, 676
Birth-weights, 49, 78, 428, 429
Blackbird, 516
Blastema, **430** ff., 443
Blastocoele, 124, 142, 169, 280
Blastocyst, 84, 367
Blastoderm, 112, 258, 265, 322, 328, 330, 420, 4
  contraction, in fish, 74
Blastokinesis, 456, 463
Blastopore, 142 ff., 162, 216 ff., 257, 324
  dorsal lip of, 103, 150 ff., 165, 175, 193, 19
    222, 275, **283**, 502
Blastula, 147, 154, 203, 339
Blindness in cave animals, 225
Blindworm, 47
Blood, 675
  and bile pigments, 648
  chemical changes in laying hen, 3
  concentration differences between materna
    and foetal, 91 ff.
  -factor in parthenogenesis, 355
  -groups, origin of, 31
  inductor, 301, 378
  presumptive, 164
  -vessel systems, axolotl, 450
  -vessel systems, mammalian, foetal an
    maternal, 598 ff.
  volume, foetal and maternal, 83 ff.
Blowfly, 57, 415, 625
Boiling of inductors, 168 ff.
Bone
  formation, 523
  heterauxesis of constituents, 555
Brachydactyly, 376
Bradyauxesis, *see* Heterauxesis
Brain
  composition, 3[illegible]
  heterauxesis of constituents, 552 ff.
  imperfect formation, 257
  inductor, 280
  tissue inductions, 180
  tissue phosphorylation, 613
Brine-shrimp, 26
Bromine content of egg, 18
Brood-pouch and respiration in sea-horses, 586
"Bull-dog" calf, 384
Butterfly, 56, 386, 392

Calcium
  heterauxesis, 544, 548 ff.
  in foetal blood, 91
  in fresh-water embryos, 39
  metabolism of laying hens, 3 ff.
Camel-cricket, 461
Cancer, **239** ff., 267 ff., 368, 386
Capsid bugs, 37 ff., 108
Carbohydrates, 564, 575 ff., 584 ff.
  absorption by embryo, 70 ff.
  metabolism in higher vertebrates, 607–16
  metabolism in insect metamorphosis, 472
  metabolism in lower vertebrates and in-
    vertebrates, 617
  metabolism in regeneration, 444
Carcinogens, 178, 243 ff., 261 ff.
Cardiac aglycones, 261 ff.
Carotenoids, 250, 652 ff.; *see also* Lipochromes
Carotin, 250, 652 ff.
Carp, 49, 541

Cartesian diver technique, 194
Cartilage
  formation, 301
  in limb regeneration, 440
  malformation, 380, 388
Casein, 4
Cassowary, 648
Caste differentiation in social animals, 309 ff.
Cat, 89, 376
Catalase synthesis, 604
Cell-division
  amphibian, 147
  and differentiation, 505, 508, 522
  and growth-promoting factor, 629
  and inductions, 177, 306
  and –SH group, 420
  bird, 332
  echinoderm, 570 ff.
  fish, 329
  insect, 435
  without nuclear division, 510
Cell metabolism, 570
Cell size in invertebrates and prochordates, 430
Cell streams, 146
Cell surface, 285
Celloidin, 305 ff.
Centrifugation
  and determination, 131 ff.
  and echinoderm respiration, 573
  disorganisation of embryo, 221 ff.
  effects on cell, 135
Cephalin, *see* Kephalin
Cephalopod egg
  dependence on environment, 34 ff., 38
  mosaic development, 140
Cerebrosides, 552 ff.
Cerebrospinal fluid, 373
Cestode
  egg constitution, 30
  respiration, 576
Chalazae, 5, 9
Chick embryo and tissues, 49 ff., 62, 175, **229**, 250, 264, 295, 350, 361, 375, 378, 390, 420, 451, 507, 522, 537, 541, 591, 598, 601, 603, 604, 610, 613, 615, 616, 635, 640, 646; *see also* Avian egg
Chimaerae, 359
Chitin, 29
Chloride heterauxesis, 544
Chlorine content of egg, 18
Chlorophyll, 644 ff.
Cholesterol, 27, 45, 86, 139, 247 ff. 262, 300, 553, 637 ff., 642, 663
Choline, 86, 211, 642 ff.
Chondrodystrophy, 380
Chorio-allantoic membrane, transplantations to, 175
"Chorion" of fish egg, 26
Choroidal fissure, 102
Chromatin, 139, 363, 509
Chromogen, 405
Chromosomes, 375 ff., 368, 672
  sex, 310, 317, 367
Ciliary polarity, 337
Cleavage, *see* Cell-division
Cleavage substances, 364
Cleidoic egg
  evolution, 33 ff.
  metabolism, 48–63, 429
Club-fingers, 377
Cockroach, 56
Cod, 653 ff.
Coelenterates
  as inductors, 172 ff.
  regulation, 134
Co-enzymes, 208, 611, 617, 636
Colchicine, 249, 262, 329, 509
Collagen, 676
Colostrum, 77
Competence, **112**, 119, 153 ff., 181, 187, 212, 231 ff., 264 ff., 281, 402, 417, 447 ff., 465, 499
Conalbumen, 8 ff.
Conductivity, electrical, 606 ff.
Constriction experiments, 103, 459, 494
Contiguity effects, **284**, 259
Copper, 12, 18, 39, 545, 651
Copulation-stimulating substances, 216
Cornea determination, 301
Cortex
  cell, 133
  of gonad, 311 ff.
Cortexin, 312 ff., 316
Cow, 80, 92, 201, 315, 378, 405, 600, 630
Crab, 51, 60, 577, 618
Creatine and heterauxesis, 544 ff.
Crop-gland, 75
Crustacea, 34, 210, 653
  tissues as inductors, 172
Crystals, liquid, 661 ff.
Ctenophore egg, 34, 133
Cuckoo, 516
Cuticle of egg-shell, 7
Cycles
  four-carbon acids, 565
  ornithine, 52, 622
Cyclopia, 141, 283, 368
Cyclostome determination, 325
Cynipids, 108 ff.
Cytochrome, 565 ff., 570, 657
Cytolysis, 656 ff.
Cytoplasm, 131 ff., 220, 352 ff., 363, 419, 632, 658

Dalcq-Pasteels theory, 220, 324, 328
Deciduoma, 84
Dedifferentiation, 525 ff.
Degrowth, 525 ff.
Dehydrogenases in embryo, 603
Determination, **99** ff., 115, 133, 430, 497 ff.
  and temperature differences, 339 ff.
  disengagement from differentiation and growth, 513 ff.
  dynamic, 146
  in birds, 330
  in cyclostomes and fishes, 325
  in echinoderm development, 476 ff.
  in insect development, 455 ff.

Determination (*cont.*)
  in mammals, 336
  in prochordates, 325
  of endoderm and mesoderm, 163
Deutectomy, *see* Yolk, spare
Deuterium, 621, 639
Diapause, 475, 516, 604, 618 ff.
  blastokinesis, 463
  respiration, 198, **578** ff.
Differential dichotomy, 112
Differentiation
  action of hormones, etc., 513 ff.
  and growth, **505** ff.
  -centre, 460 ff.
  dependent, **103** ff., 106, 110, 139
  disengagement of, 514, 522
  energetics of, in echinoderm egg, 574
  -inhibiting factors, 360
  self-, 103, 112 ff., 139 ff., 282 ff., 514
  self-, "Bedeutungsfremde", 157, 163, 409
  without cleavage, 510 ff.
Diffusibility of inductors, 312, 418, 454
Diminisher, 139
Dipeptidase of blastema, 446
Diploidy, 357 ff., 434
"Dissection" of metabolism, 520
Dissociability, 505
Dog, 91, 92
Dogfish, 38 ff., 321 ff.
Dominance, 404
Dosage of evocator, 182
"Double assurance", 293, 308
Double refraction, *see* Birefringence
Dove, 63, 393
Duck tissue as inductor, 350
Dyes, 189
  reduction method, 203, 335, 497
  vital, 84, 133
"Dynamic structure", 658, 675 ff.

Ear
  inductor, 280
  vesicle, 295, 301, 666
  vesicle inductor in chick, 335
Earthworms, double gastrulation, 228
Echinochrome, 29, 215, 568, 657
Echinoderms, 134, 136, 206, 220, 509, 514, 642, 671, 673
  development, **476–502**
  egg, regulation, 131
  egg, constitution, 28, 34
  egg, dependence on environment, 38
  egg secretion, 214
  parthenogenesis, 356
  parthenogenetic merogony, 362
  respiration, 562–577
  respiratory enzymes, 604
  –SH metabolism, 421 ff.
Ectoderm, 113 ff., 124, 142, 156 ff., 169, 176, 268, 273, 277
  balls, 167, 278, 280
  competence, 281
  expansion, 146
Ectodermisation, 477

Egg-albumen, 177 ff.
Egg
  aquatic, dependence on environment, 38
  inorganic constituents, 17
  ovarian, as inductor, 171
  -secretion, 214 ff.
  -shell, 4 ff., 7, 21, 392, 644
  -white constitution, 5 ff.
  -white enzymes, 73
  -white, physical properties, 10 ff.
  -white, protein metabolism, 620
"Elephant" embryos
  in amphibia, 275
  in mammals, 372
Eidogens, 279
Emancipation, 287
Embryo-extract, 628 ff.
Emphysema, 388
Endocrines
  and carbohydrate metabolism during chick development, 609
  and genetic abnormalities in birds, 380
  and reproductive cycle in fishes, 43
  function of foetal, 87
  See also Hormones
Endoderm, 142 ff., 163 ff., 282
Endodermisation, 477
Endometrial mole, 84
Energy-sources, succession of, 603
Entelechy, 119
Environment and egg, 32
Enzymes
  and genes, 390
  in amphibian embryo, 625
  in cell protoplasm, 657
  in diapause, 581
  in human colostrum, 77
  in marine and invertebrate eggs, 627
  in placenta, 85, 89 ff.
  in yolk-sac, etc., 70 ff.
  phosphorylation, 611
  proteolytic, 208
  respiratory, 603 ff.
Epiboly, 146
Epidermis, 144, 156 ff., 169, 283, 437
Equilibria, unstable and stable, 114
Ergosterol, 24, 247 ff., 639
Erythrocytes, 355, 378, 648 ff.
Evocation, 125, 188, 271
Evocators, 169, 173, 175 ff., 189, 205, 219 ff., 234, 243 ff., 255, 257, 276, 302, 450, 462, 670
Evolution, 33 ff., 59, 500, 679
Exchange transplantations, 148, 150
Exo-embryo, 159, 273
Exo-gastrulation, 159 ff., 236, 495
Expressivity, 401
Eye
  abnormalities, 396, 398
  pigmentation, 407 ff., 409
  transplantation in axolotls, 345 ff.
Eye-cup
  induction, 105, 291
  suppression, 225

Facet-forming substance, 398
Falcon, 542
Fat
  absorption by embryo, 70 ff.
  -bodies and nuclear inductors, 411
  heterauxesis, 541 ff.
  in blood of laying hen, 3
  in cleidoic egg, 48 ff., 62 ff.
  in egg-yolk, 16 ff.
  in insect metamorphosis, 471 ff.
  in placenta, 86
  metabolism of egg, **637** ff.
Fate-maps, 144 ff., 283, 321 ff.
Fatty acids as inductors, 182 ff.
Fermentation inhibition, 520, 566, 596
Fertilisation
  and echinoderm determination, 488
  and induction, 214 ff.
  and respiration, 563, 569 ff.
  immunological interpretation of, 214
Fertilisin, 214 ff., 572 ff., 587
Fibre-molecules, 658
Field concept, 127 ff.
Fishes
  blindness, 225
  determination, 325 ff.
  egg, carbohydrate metabolism, 618
  egg, dependence on environment, 34
  egg, organisers, 329
  egg, protein metabolism, 624
  parental care, 32
  tissues as inductors, 173
Fishes, elasmobranch
  constitution of eggs, 26 ff.
  dependence of embryo on environment, 39 ff.
  heterauxesis, 541
Fishes, teleostean, 606, 620, 634, 653
  constitution of eggs, 6, 25 ff.
  development, 326
  evolution of ovoviviparity, 46
  heterauxesis, 541
  oestrogens, 316
  presumptive parts, 321
  respiration of eggs, **584** ff.
Flavines, 208, 654
Fluorine in avian egg, 18
Fly, 386
  Blow-, 57, 415, 625
  Flesh-, 57
  Fruit-, 361, 395, 398
  Fruit-, eyeless, 376
  House-, 463
Form and Matter, xv
Formative movements
  in amphibian egg, 145
  of ectodermal isolates, 168
Four-carbon acid oxidation cycle, 565
Fowl, 65, 271, 428, 429, 526, 523, 601, 606; *see also* Avian egg
Freemartin, 313
Frog, 33 ff., 39, 47 ff., 103, 105, 136, 193, 219, 255 ff., 286, 617, 636
  gastrulation, 141
  regeneration, 439
Frog (*cont.*)
  regeneration and cancer, 240
  respiration, gastrula and adult, 201
  sex-hormones, 313, 316
  thyroxin, 264
Fructose, 616

Galactogen in molluscan egg, 29
Galls (plant), 67, 105 ff., 368
Gall-gnat, 107
Gall-wasp, 107 ff.
Gastropod, 29, 39, 58 ff.
Gastrula, 147, 273, 339
  and primary organiser, 150 ff.
  metabolism, 190
  respiration, 193–203
  –SH groups, 203
  staining, 144
Gastrulation, 101 ff., 276, 322, 363, 603, **673**
  amphibian, 141 ff.
  amphibian and avian, 334
  anomalous, 218
  avian, 331
  cyclostomes and fishes, 326, 329
  echinoderm, etc., 575
  inhibitors, 204
Genes, 24, 230, 271, 340–419, 474; *see also* separate index
Genetic boundaries, 341
Germ-cells, 310
Germ-layers, 150
Gills
  formation, 303
  modification, 47 ff.
  opercular perforation, 308 ff.
Glands, *see* Endocrines and Hormones
Glucolysis, 521, 613 ff., 617
Glucose, 92, 518, 608, 618
Glutathione, 85, 420 ff., 443, 544 ff., 582 ff., 614
Glycerides, passage through placenta, 93
Glycine in chondrodystrophy, 380
Glycogen
  and evocator, 205
  as evocator, 278
  heterauxesis, 544 ff.
  in amphibian gastrula, 190 ff.
  in chick embryo, 334
  in eye-cup, 300
  in hen's egg, 608 ff.
  in insect metamorphosis, 471
  in lower animals, 617 ff.
  in molluscan eggs, 29
  liver-, 523
  producing neural inductions, 179 ff.
  storage in placenta, 85
Glycolysis, 209, 615
  in chick embryo, 593 ff.
  in frog gastrulae, 193 ff.
  in mammalian embryo, 596
  inhibition, 265, 519
Goat, 91, 92, 599
Goldfish, 75
Gonads, 310
  pigmentation, 417

Gradient
  animal-vegetal in amphibian egg, 216 ff.
  animal-vegetal in echinoderm egg, 477 ff.
  concept, 121, 129, 220
  echinoderm, 501 ff.
Grasshopper, 27, 36 ff., 49 ff., 56, 198, 358, 463, 578, 580, 581, 604, 618, 626
Grass-snake, 35
Grebe, 36
Grey crescent, 216 ff., 257, 276
Growth
  as separate factor, 505–562
  -promoting factor, 350, 628
Guanine, 50
Guinea-pig, 91, 313, 368, 375, 377, 390, 405, 616 ff.
Gynandromorphs, 317, 408
Gynogenesis, 356
Gyno-merogons, 358
Gypsy-moth, 317

Haematin, 19
"Haematogen", 19
Haematopoiesis, 650
Haemoglobin, 19, 22, 589, 594 ff., 598 ff., 644, 648
Haemolymph, pupal, 474
Haemophilia neonatorum, 92
Hallochrome, 406, 568
Halogens in avian egg, 18
Haploidy, 352, 357 ff., 434
Hare-lip, 507
Harmine, 343
Hatchability, 18, 20 ff., 36
Hatching
  and genes, 388
  proteases, 627
  weights, 49
Hawk-moth, 56, 172
Head
  abnormalities, 370
  organiser, 272 ff., 368
  region, 274
Heart
  as lens-inductor, 299
  -beat of transplants, 347
  induced by adult tissues, 280
  stoppage by interspecific toxins, 351
Heat
  -production of insect pupae, 474
  -production in fish eggs, 585
Hen, 3 ff., 64, 651, 652; *see also* Avian egg
Hensen's node, 323
Herkunftsgemäss, *see* Determination
Heterauxesis, 343 ff., 524, 531 ff., 616
Hetero-auxin, 185
Heterotaxia, 65
Hibernation, embryonic, 516
Hippomanes, 81
Histogenesis, 541
Histogenetic substances, 359
Histolysing activity of blastema, 446
Histotrophe, 42, 75, 81, 85
Homoiogenetic induction, 154, 174, 267, 307, 33
Homologous space and time, 561
Homology, biological, and inductors, 320
Hormones
  A-, 416
  activity compared with neural inductions, 184
  and avian "milk", 76
  classification, 289, 418
  differential action on differentiation and growth, 513
  effect of genes on, 390
  effect on chick liver, 609
  in amphibian regeneration, 439 ff.
  in axolotl metamorphosis, 450
  in insect metamorphosis, 464 ff., 467
  in plant growth, 106 ff.
  placental, 87
  sex-, 252, 261 ff., 310 ff., 639
Hornet, 56
Horse, 25, 263, 315, 355, 429
House-fly, 57
Humidity, effect on chick development, 65
Hybridisation, 352 ff.
Hybrids, false, 356
Hydatids of Morgagni, 311
Hydration, *see* Water
Hydrocarbons
  and neural induction, 401 ff.
  as evocators, 178
  carcinogenic, 244, 266
  in silkworm eggs, 28
Hydrocephaly, 373
Hydrocoele, 499
Hydropyle, 37
Hymenoptera, parasitic, 228
Hypocalcaemia, 5

Imaginal discs, determination, 468 ff.
Immunity, embryonic
  inheritance, 30 ff.
  placental transmission, 90 ff.
Implantation
  heteroplastic, 171
  method, 152
Inactive evocator complex, 205 ff.
Incubation time, 49
Individuation, **125**, 187, 271, **286**, 343
  field, 126 ff., 228 ff., 239 ff., 257, 275, **278** ff., **281** ff., 286, 379
Induction, 104, 124, **168** ff., 173, 183, 280; *see also* Organisers
Inductive power, 152 ff., 282
Inductors, 116 ff., 290 ff., 332, 447, 464
Inhibition of individuation field, 282
Inhibitors, 204, 465, 498, 563 ff.
Inorganic substances
  in avian egg, 17
  in insect metamorphosis, 475
Insects
  abnormalities and genes, 377
  development, 455–76
  egg dependence on environment, 34
  egg maturation, 6, 27

Insects (*cont.*)
  egg metabolism, 642
  egg organisation, 172 ff.
  egg types, 60 ff.
  egg, water intake, 36 ff.
  excretion, 56 ff.
  gall-production, 107 ff.
  melanin-forming power, 406
  polyembryony, 228
  wing patterns, 403
Insulin, 608 ff.
Intersex, invertebrate, 317 ff.
Invagination, 142 ff., 221, 223, 322, 326, 674
Iodine
  effect on amphibian metamorphosis, 448, 450, 453
  in avian egg, 18
  -value of fatty acids of various eggs, 30
Iris, 295 ff., 523
Iron in avian egg, 18 ff., 651
Irradiation
  and dissociability, 519 ff.
  effect on regeneration, 440 ff.

Jelly, amphibian egg, function of, 25

Kathepsin, 71 ff., 444 ff., 623
Kephalin, 17, 180 ff., 638
Keratin, 27
Ketosis, 62 ff.
Kidney as inductor, 279 ff., 613; *see also* Pronephros
Kinetics of gene action, 392
Kynurenin, 410 ff.

Lackey-moth, 50
Lactation, effect on glutathione in young, 426
Lactoglobulin, 4
Lamprey, 321, 451
Larval compression, 32
Lead, 18, 545, 556
Lecithin, 4, 16, 620, 638
Leech, 51
Lens, 105, 169, 175, 224, 291, 329, 335
Lethality, 365, 402
Leucocytes, 355
Limb-bud
  determination, 665
  development and vitamin A, 228
  induction, 305 ff.
  induction, interference with, 376 ff.
Limbs
  formation, 287
  regeneration, 430 ff.
  transplantation, in axolotls, 344
Lipases in egg and yolk-sac, etc., 71 ff.
Lipin metabolism, 637 ff.
Lipochromes, 23, 250, 652 ff.; *see also* Carotenoids
  -protein complexes, 210
Lipoids
  in diet, effect on eggs, 23
  in elasmobranch liver, 44 ff.
Lipoids (*cont.*)
  in placenta, 86
Lipo-phanerosis, 206
Lithium
  effect on sea-urchin eggs, 485 ff., 488 ff., 492 ff.
  production of cyclopia by, 283
  suppression of notochord by, 226
  susceptibility of organisers to, 276 ff.
Liver
  and foetal atrophy, 65
  as inductor, 279 ff., 298 ff., 523, 613, 618, 640, 649, 651
  composition of fish-, in relation to reproductive cycle, 44 ff.
  development, 174, 178
  reduction during fish pregnancy, 43 ff., 46
Livetin, 16, 27, 620, 672
Lizard, 36, 61, 223, 225, 439
Lobster, 653
Locust, 619
Lung-fish respiration, 586
Luteosterone, *see* Progesterone
Lysozyme, 73

Maggots and wound-healing, 57
Malaria equations, 114
Malpighian tubules
  and gall production, 109
  and nuclear inductors, 411
Mammals
  and ascorbic acid, 616
  and pyrrol derivatives, 647
  and sex-hormones, 313 ff.
  and tumours, 269
  determination, 336
  development, 541
  effect of carcinogens, 265
  effect of thyroxin, 264
  egg, antigens and antibodies, 30
  egg, dependence on maternal organism, 34
  egg organisation, 172 ff.
  embryonic excretion, 623
  embryonic respiration, 595 ff.
  fat metabolism, 640
  fate-map, 325
  nutrition, 77
  pregnancy, 616
  respiratory enzymes, 604
  species-specificity, 340
  tissues, as inductors, 280
  ureotelic metabolism, 51 ff.
Mammary carcinomata, 253
Man, 89, 91, 172, 227, 315, 375 ff., 599, 609, 616
"Marking time" and "catching up", 346, 347, 449, 589
Mathematical approach to embryonic growth, 531
Maturation of egg, 3 ff.
May-fly, 56
Mealworm, 539, 541 ff.
Medulla, of gonad, 311 ff.
Medullarin, 312 ff., 316
Melanin, 405, 655

Melanism, 405
Melanoma, 386
Melanophores, 278
Merogony, 357
Mesoderm, 142 ff., 150, 154 ff., 158 ff., 163 ff., 276, 310, 674
Mesoforms, 663 ff.
Metabolic rate, 600 ff.
Metabolism, 180 ff., 257, 262 ff., 387 ff., 471 ff., 494 ff., 506, 514 ff., 517 ff., 523, 570, 572 ff., 578 ff., 593, **605–56**
Metamorphosis
  accelerators of insect, 262 ff.
  amphibian, **447** ff.
  echinoderm, 476, 498 ff.
  insect, 455 ff., 463 ff., 470
Metathetely, 466
Micromanometers, 197
Micromeres, 480 ff., 488 ff., 500
Milk and colostrum, of mammals, **75**
"Milk", avian, 75, 310
Minnow, 230, 584
Mitosis, *see* Cell-division
Mitotic rate, *see* Cell-division
Modulation, 279, 506, 514, 529
Molecular orientation and contractility, 27, 670
Molluscs
  egg, anisometric protein molecules in, 671
  egg, constitution, 29
  egg, intake of calcium, 39
  egg, mosaic quality, 131, 134, 140
  egg, respiration, 562 ff.
  egg, secretion, 215
  excretion, 215
  testis effect in parthenogenesis, 355
  tissues, as inductors, 172 ff.
Morphogenetic stimuli, 103 ff.
Mortality, embryonic
  avian, 63 ff.
  other animals, 65
Monotremes, ureotelic metabolism, 56
Monsters, 370
Mosaic eggs, 99, 116, 131 ff., 456, 676
Mosaics
  genetic, 408
  in aphids, 117 ff.
Mosquito, 6
Moth, 37, 56 ff.
Moulting, insect, 464 ff.; *see also* Insects
Mouse, 91, 92, 172, 245, 253, 267, 271, 361, 367 ff., 388 ff., 598, 632
Mucoprotein, 10
Mullerian duct, 263, 311, 320
Muscle, 164, 174, 441, 613, 643, 658, 661, 675
  and genes, 390
  -cell growth in trout, 512
  inductor, 280
Mustard gas and competence, 265
Mutations, and tumours, 271
Myoplasm, 136 ff.
Myosin, 119, 671

Nakedness, 378
Nasal placode, 297, 306
Neighbourwise, *see* Determination
Nematodes, 108, 522, 618
  in muscle fibre, 659
  mosaic eggs, 138
  respiration, 576
Nemertines, larval compression, 33
Neoplastic growth in reptiles and amphibia, 240 ff.
Neoteny, 450
Nerve, 643
Neural axis, 156, 172, 326
Neural induction, 116 ff., 157, 177 ff., 211, 281, 333, 501
  and hydrocarbons, 401 ff.
  and -SH groups, 204
  and temperature, 338
  by mechanical injury, 188
Neural plate, 113, 128, 145, 153 ff., 162, 174, 268, 283, 667
Neural tube, 257, 284
Neural tissue, 154, 177
Neurogens, domain of specificity, 261 ff.
Neurula, 155, 163, 274, 281, 288, 304
Neurulation, 670
Neutron bombardment of eggs, 230
Newt, 101, 103, 113, 125, 141, 147, 172, 175 ff., 219, 237, 240, 297, 441, 448
Nidicolous habit, 76
Nidifugous habit, 76
Nitrogen
  amino- in foetal and maternal blood, 92
  -excretion, 50 ff., 621 ff.
  heterauxesis, 544
  isotopic, 621
  metabolism in tail resorption, 452
  storage in embryo and membranes, 68 ff.
Nitrophenols, action on echinoderm egg, 570
Nitroprusside reaction, 420 ff.
Nose, *see* Nasal placode
Notochord, 145, 174 ff., 226, 257, 280, 319
Nuclear division, 455, 508 ff.
Nuclear inductors, 408 ff.
Nuclear metabolism, 418 ff.
Nucleic acids, 636, 658
Nuclein metabolism, 632 ff.
Nucleotides, 631 ff.
Nucleus
  in development, 351 ff., 364
  microchemistry of, 418
Nutrition
  embryonic, 66
  maternal, effect on yolk, 4
  maternal, in mammalian development, 77

Octopus, 39 ff.
Oedema, 357
Oestrogens, 87, 178, 252 ff., 261 ff., 315
Oestrone, 84, 252, 262 ff., 313, 315 ff., 639
Oocyte
  maturation, 3 ff.
  -SH groups, 203
Opercular perforation, 308

Organ
  differentiation, 290
  districts, 430 ff.
  formation, 286
Organic solvents, effect on evocator, 177
Organisation and energy, xv
Organisation-centre, 151
  of amphibian gastrula, 203
  of chick embryo, 335
  in isolation, 283
Organisers, 103, **110 ff.**, 124, **148–163**, 165–188, 334
  and cancer, 239 ff.
  and fate-maps, 320 ff.
  and genes, 340 ff.
  and lethal genes, 365
  and recapitulation, 319 ff.
  excess, 231 ff.
  inhibition, 222 ff.
  phenomena in echinoderms, 500 ff.
  phenomena in insects, 456
  second-grade, 290 ff., 300 ff., 329, 335
  second-grade and interference by genes, 373, 375 ff.
  second-grade and temperature, 340
Organogenesis, 514
Ornithine cycle, 52, 521, 622
Ortsgemäss, *see* Determination
Osmotic pressure in eggs, 11 ff., 39, 606
Osteoblasts, 523, 555
Otocephaly, 368 ff.
Ovarian teratomata, 231 ff.
Over-ripeness of frog eggs, 257
Oviducts of laying hen, 3
Oviparity, 34, 41, 46, 51
Ovoalbumen, 7 ff., 90, 620, 672
Ovoglobulin, 7 ff., 671
Ovomucin, 7 ff.
Ovomucoid, 8 ff., 620
Ovotyrin, 15
Ovovitellin, 4
Ovoviviparity, 33 ff., 38, 47, 56 ff.
Oxidation in mammalian embryo, 597
Oxygen
  consumption in frog gastrula, 199
  effect on differentiation, metabolism, etc., 522
  See also Anaerobiosis; Respiration

Palisade inductions, 177 ff.
Pantothenic acid, 214
Paracrystalline state, 661 ff.
Parathyroid hormone, 5
Parental care of young, 32 ff., 46 ff.
Parnas reaction, 615
Parthenogenesis, 215, 352, 575, 583
Parthenogenetic merogony, 362
Pasteur effect, 596
Penetrance, 401
Peristeridae, wild, 64
Periwinkle, 58 ff.
Peroxidase, 582
Pflügerian hermaphroditism, 315
*p*H
  and echinoderm egg respiration, 565
  differences in egg regions, 133, 606, 668
  in insect pupa metabolism, 475
  in regenerating tissues, 442
  in tail resorption, 452
Pheasant, 64
Phenocopies, 370, 392, 402
Phenocritical periods, 402
Phosphagen, 615, 617
Phosphatides, 16 ff., 86, 91, 637 ff.
Phosphorus, 3, 91, 475, 545, 548, 555 ff., 617, 619
Phosphorylation, embryonic, 610
"Physiological time", 561
Pig, 92, 620, 313, 378, 651
Pigeon, 63, 75 ff., 350, 428
Pigeon milk, *see* "Milk", avian
Pigment
  complexes, 208 ff.
  metabolism, **643 ff.**
Pigmentation patterns, 403
Pike, 75
Pipe-fish, 47
Pituitary, 43, 88, 317, 380, 390, 450, 454, 559
Pituitary hormone, 450, 454
Placenta, 31, 79 ff., 85 ff., 90 ff., 315, 550 ff., 598, 613, 616
  types, classification of, 81
Plaice, 49 ff., 541
Plant
  hormones, 212
  implantations, 175
  poisons, 250
Plasmalogen, 189 ff.
Plasmodium, 672
Pleiotropic genes, 388, 416
Pluripotency, 100, 122, 228, 435 ff.
Pluteus, 477 ff.
Polarity, 497, 656 ff., 667
"Polonaise movement", 331
Polychaete worms, *see* Worms
Polydactyly, 373, 376 ff.
Polyembryony, 228 ff.
Polysaccharides, 29, 618, 658
Porphyrins, formulae, 644 ff.
Potassium
  effect on cleavage, 510
  effect on respiratory rate of echinoderms, 487
  heterauxesis, 544
Precipitins, 349
Presumptive fate, 102
  deviations in echinoderms, 480 ff.
Presumptive regions, 143 ff., 286
Primitive streak, 275, 323, 333
Pristane, 45
Prochordates, determination, 325
Progesterone, 84 ff., 87, 252, 262
Prolactin, 76
Pronephros, 163, 280, 319 ff.
Prospective potency, 100, 282 ff.
Prospective significance, 100, 282 ff.

Proteases
  hatching, 627
  in egg and yolk-sac, etc., 71, 623
  in regeneration, 444
Proteins
  absorption by embryo, 70 ff.
  and species-specificity, 348, 350
  catabolism in echinoderm egg, 487
  complexes, 206 ff., 269, 316, 423
  diet, effect on avian eggs, 22
  egg-white, 7 ff.
  gastrular, 203
  metabolism, 603
  metabolism in cleidoic eggs, 48
  metabolism in higher vertebrates, 619 ff.
  metabolism in insect metamorphosis, 471 ff.
  metabolism in lower animals, 624
  molecules, 119, 265, 658, 671
  yolk-, 3 ff., 15
Proteoses, 630
Prothetely, 239, 466
Protoplasm, 120 ff., 656, 659, 661, 672
Pseudencephaly, 371, 373
Pterines, 649 ff., 654 ff.
Pupation, 56, 409, 467, 474 ff.
Purines, 634 ff.
Pyrrol derivatives, 644 ff.

Quail, 64

Rabbit, 30, 31, 86, 89, 91 ff., 171, 270, 336, 350, 376, 378, 390, 425, 429, 509, 595, 604, 611, 617
Radiation, γ-, effect on growth, respiration, etc., 519; *see also* X-radiation, Irradiation, Ultra-violet, etc.
Rat, 91, 263, 265, 336, 350, 378, 388, 390, 420, 517, 520, 524, 541, 555, 559, 595, 598, 600, 609, 617, 632
Rate genes, 392, 405
Ray, 26, 27
Recapitulation phenomena, 54, 319 ff., 348
Recuperation, 442
Regeneration, 240, 430, 442
Regional differentiation, 124, 273, 277
Regression, 525 ff.
Regulation, 355
Regulation eggs, 131, 140, 456
Reptiles
  and cancer, 240
  egg, antigens and antibodies, 30
  egg, constitution, 24 ff.
  egg, dependence on environment, 34
  egg, water supply, 35 ff.
  neoplasmoid blastoderms, 258
  ovoviviparity and viviparity, 61
  sex-hormones, 314 ff.
  sex reversal effects, 314
  tissue as inductor, 173, 280
  uricotelic metabolism, 53 ff.
Resin acids domain, 262
Respiration, **562–605**
  and protein metabolism, 621
Respiration (*cont.*)
  inhibition, 517 ff.
  of frog gastrulae, 193–203
  of grasshopper and sea urchin, 198 ff.
  of nucleus, 419
Respiratory quotients
  and cleavage, 512
  during diapause, 581
  during insect metamorphosis, 472 ff.
  of amphibia, 587
  of chick embryo, 591 ff.
  of echinoderm egg, 486, 575 ff.
  of embryonic membranes, 70
  of frog gastrula, 193
  of Sauropsida, 591
  of teleostean fish eggs, 584
  of worms, 576 ff.
Retina, 613
Reunition of sponges, 528
Royal jelly, 310

Sacral teratomata, 236
Salamander, 39, 47, 49 ff., 174, 313, 437, 439, 6
Salmon, 6, 26, 38, 49, 616
Sandgrouse, 36
Saponins, 250
  aglycones domain, 262
Sarcomata, 259, 269
Sauropsida
  amnion formation, 146
  fate-map, 321 ff.
  growth without differentiation, 507
  organiser, 223
  suppression, 223
  respiration of eggs, 590
"Sausage" embryos in echinoderms, 497
Sawfly, 37, 107 ff.
Schultze's experiment, 276
Sea-horse, 46 ff., 586
Sea-urchin, 28, 34, 49 ff., 99, 104, 121, 19 364, 422, 478, 493 ff., 500, 517, 606, 618 ff 625, 633, 636, 656 ff., 667
Selfwise, *see* Determination
Seminoma, 238
Senescence, 559
Sex-chromosomes, *see* Chromosomes
Sex determination, 310 ff.
Sex-hormones, *see* Hormones
Sex-reversal in vertebrates, 313 ff.
–SH groups, 136, 203, 419 ff., 267
Shark, 27, 45
Sheep, 92, 229, 376
  -blowfly, 49 ff., 57
Shrimp, 398, 407
Side-plate mesoderm, 153
Silkworm, 28, 49 ff., 56, 65, 367, 516, 583, 62
  moth, 475, 607, 618, 625, 653
Skin abnormalities, 378 ff.
Snail, 29, 58 ff.
Snake, 61
Somites, 145, 163, 174 ff.
Spatial axes, of organs, determination of, different times, 665 ff.

pecies-specificity, 31, 348, 415
pecificity, biological, of chemical compounds, 261 ff.
perm, 218, 356
ponge, 528
pringtail, 36
qualene, 29, 45, 250
quid, 38 ff., 49 ff., 618
tability landscapes, 114
teroids
  as evocators, 178, 186
  formulae, 245 ff.
"Sterol bumps", 177, 258
terols
  as carcinogens, 244 ff.
  domain, 262
  in egg-yolk, 16 ff.
  metabolism, 235, 258, **637** ff.
tick insect, 243
tickleback, 75
tilbene derivatives, 185, 256
Stimulating substances, biological, activities of, compared, 184
Sugar in blood of laying hen, 3
uprarenals, *see* Adrenal
wift, 75
Symmetry, bilateral, 217, 283
Syndactyly, 373, 376

Tachyauxesis, *see* Heterauxesis
Tadpole, *see* Frog
Tail
  -buds, presumptive, 328
  development, 148
  inductor, 280
  organiser, 272 ff.
  organiser, interference with, 374
  resorption in amphibia, 451
Tars, 244
emperature effects
  on amphibian respiration, 589
  on butterfly wing pattern, 392
  on chick development, 65
  on cleavage rate, gastrulation, etc., 575
  on echinoderm respiration, 564
  on heterauxesis, 557
  on morphogenetic processes, **336** ff.
  on phenocopies, 395
Teratomata, 231 ff., 514
Terrapin, 35
Testis pigmentation, 416 ff.; *see also* Teratomata
Testosterone, 186, 252, 262, 313 ff., 317
Tetany, 5
Thermophenes, 395 ff.
Thixotropy, 658, 672
Thorium, 495
Thymus, 4
Thyroid
  and elasmobranch liver, 43
  and genes, 380, 390
  effect on amphibian metamorphosis, 264, 448
  effect on axolotl, 450
  effect on other vertebrates, 451 ff.
Thyroxin, 116, 262 ff., 308, 448, 450 ff., 583
Tissue affinities, 284
Toad, 33, 39, 147, 351
  fate-map, 321
  regeneration, 440
  sex-hormones, 312
  venom, 250
Tobacco, 495
Torpedo, 38
Tortoise, 54
Toxins, interspecific, 350
Transplantations
  exchange, 148, 150
  heteroplastic, 156, 171
  homoioplastic between younger and older animals, 346, 439, 449, 454, 460, 589
  non-embryonic, 260
Trophoblast, 84, 89, 146
Trout, 25 ff., 33, 38, 49 ff., 73, 75, 230, 322, 338, 451, 512, 624, 634
Tumours, 270, 385 ff., 393, 613, 632
Turacin, 644
Turkey, 64
Turtle, 24, 35, 38, 49, 54, 56
Twinning, 218, 228 ff.
  in mosaic eggs, 140
Tyrosinase, 406

Ultra-violet irradiation
  of blastoderm, 334
  of embryo, 222
  of larvae, 440
  of yeast, 631
  producing twinning, 230
Uranium, 495
Urea, 50 ff., 625
Urechrome, 575
Uric acid, 50 ff., 625
Uricase, 52, 54
Uricotelism and ureotelism, 50 ff.
Urinogenital system, 319
Urodeles and cancer, 240
Uterine fluid, composition in elasmobranchs, 40 ff.
Uterine wall, elasmobranch, 42 ff.

Ventral ectoderm, 193, 197
Vertebrate axis appearance, 148 ff.
Virus
  plant viruses, 672
  theory of cancer, 267
Viscosity of protoplasm, 661
Vitamins, 17
  A, 17, 23, 26, 77 ff., 210, 227, 249, 653
  B, 391
  B_1, 12, 17, 23, 26, 208 ff., 227
  B_2, 12, 17, 23, 26, 208 ff., 389, 654
  B_3, 214
  B_6, 208 ff.
  C, 17, 136, 211, 266, 616
  D, 17, 23 ff., 250, 262, 280, 284
  E, 17, 24, 211, 264 ff.
  K, 265

Vitamin (*cont.*)
  P-P, 17
Vitellin, 3ff., 15ff., 26, 620, 672
Vitelline globules, 217
Vitelline gradient, 276
Vitelline membrane, 11ff.
Vitellomucoid, 16, 620
Viviparity, 34, 41, 43ff., 53, 60

Water
  heterauxesis, 540, 560
  hydration of chick embryo's membranes, 68
  supply to eggs, 34ff., 63ff.
Weckung, 156, 490
Wings (insect) and genes, 392, 399ff.
Wolffian duct, 263, 311, 319ff.
Wolffian regeneration, 295ff., 340
Woodlouse, 541
Worms
  annelid, 259
  egg, constitution, 30, 131
  gall-producing, 108
  gephyrean, 318
  planarian, 422
  polychaete, 133, 210, 215
  polychaete and tumour growth, 260
Worms (*cont.*)
  respiration, 562ff.
  tissue as inductor, 173
Wound-healing, 632

Xanthophyll, 652ff.
Xenoplastic transplantations, 349
X-radiation
  and growth inhibition, 213
  effect on eggs, 225
  effect on growth, respiration, etc., 519

Yeast, 214, 267, 411, 424, 495, 515, 520, 568,
Yolk
  absorption, 349
  and diapause, 581ff.
  constitution, 4ff., 11ff., 14, 653
  endoderm, respiration, 201, 283
  enzymes, 70ff.
  pigments, 22ff.
  proteins, 3ff.
  protein metabolism, 620
  removal from embryo, 328
  -sac growth, 66ff., 82ff.
  spare, 73
  utilisation, 73

INDEX OF ANIMALS

PROTOZOA

noeba, 657
do caudatus, 521
hilomonas, 588
ramoecium, 421
irostomum, 659

PORIFERA

rtularia, 134
candra, 134

COELENTERATA

(*a*) HYDROZOA

rysaora, 140, 527
ydra viridis, 75, 172, 242
elia, 669

(*b*) CTENOPHORA

roe, 34, frontispiece, opp. 132
— *ovata*, 131

PLATYHELMINTHES

(*a*) TURBELLARIA

anaria dorotocephala, 525
olychoerus carmelensis, 28

(*b*) CESTODA

iphyllobothrium latum, 30, 576
hinococcus granulosus, 108
oniezia expansa, 576
aenia crassicollis, 108
eniarhynchus saginatus, 30
iaenophorus lucii, 576

NEMERTINEA

rebratulus, 667
eus, 527

NEMATODA

caris, 139, 660, 668, 672
— *megalocephala*, 577
ichurus trichiura, 576

ANNELIDA

(*a*) OLIGOCHAETA

umbricus terrestris, 355
— *trapezoides*, 228

(*b*) POLYCHAETA

mphitrite, 511 ff.
ricia, 139
ratocephale osawai, 210, 652
haetopterus, 132 ff., 140, 189, 363, 510 ff., 519, 530, 563, 574, 627
erpobdella, 134 ff.
Hydroides hexagonus, 28
Nereis, 75, 136 ff., 260, 563 ff.
— *dumerilii*, 133
— *limbata*, 133
Podarke, 422, 511
Pomatoceros, 134 ff., 514
Sabellaria alveolata, 116, 140, 456
Tubifex, 627
— *rivulorum*, 140

(*c*) HIRUDINEA

Hirudo medicinalis, 51

(*d*) GEPHYREA

Bonellia, 318
Urechis caupo, 565, 572 ff., 603, 626 ff., 634, 650, 668

ECHINODERMATA

Anthocidaris, 509
Arbacia, 28, 134 ff., 362 ff., 423, 478, 496, 508, 518 ff., 564 ff., 572 ff., 627, 642, 660, 668
— *punctulata*, 28, 563
Asterias, 568
— *glacialis*, 563
Clypeaster, 509
Dendraster excentricus, 364, 419, 575, 627, 674
Diadema, 509
Echinarachnius, 496, 627
Echinometra lucunter, 28, 626
Echinus, 34, 99
— *microtuberculatus*, 574
Lytechnis, 575
Paracentrotus (*Strongylocentrotus*), 137, 364, 478, 508 ff., 572, 575, 626, 635
— *lividus*, 49, 50, 479, 563 ff.
Psammechinus, 140, 627
— *miliaris*, 563 ff.
Sphaerechinus granularis, 363, 563

ARTHROPODA

(*a*) CRUSTACEA

Artemia salina, 26, 364, 659
Astacus fluviatilis, 52, 54, 509
Carcinus moenas, 577
Daphnia magna, 60
Emerita analoga, 34, 634
Eriocheir sinensis, 51
Gammarus, 398, 414
Maia squinado, 634, 653
Niphargus, 225
Oniscus murarius, 543
Palaemon, 377
Paralithodes kamschatica, 443
Polyphemus, 60

(*b*) PROTOTRACHEATA

Peripatopsis, 56

(*c*) INSECTA

Acidalia virgularia, 417
Aëdes argenteus, 36
Agelastica alni, 457
Anisolabis, 466
Antheraea pernyi, 56 ff., 472, 626
Apis mellifica, 457
Apotettix eurycephalus, 391
Arphia sulphurea, 27
Asterolecanium variolosum, 108
Attacus polyphemus, 473
Blatella germanica, 56
Blatta orientalis, 28, 56
Bombyx mori, 34, 49 ff., 416, 472, 496, 583, 642
Bruchus, 457, 460
Calligrapha, 457
Calliphora, 398, 410 ff., 457, 467
— *erythrocephala*, 56, 410, opp. 464
Camnula pellucida, 36
Camponotus, 457, 460
Carebara, 429
Celerio euphorbiae, 361
— *gallii*, 361
Chermes, 109
Chortophaga viridifasciata, 579, 583
Colias philodice, 653
Conotrachellus nenuphar, 368
Cryptochaetum grandicorne, 584
Culex pipiens, 6
Deilephila euphorbiae, 56, 172, 475, 583
Dissosteira carolina, 27
Dixippus, 377, 466 ff.
— *morosus*, 343
Drosophila, 28, 227, 230, 242, 318, 362, 366 ff., 376 ff., 385 ff., 390 ff., 402 ff., 413 ff., 457, 467, 473 ff., 510, 634 ff.
— *azteca*, 415
— *melanogaster*, 472
— *pseudobscura*, 415, 472
Dryophanta erinacei, 109
Dytiscus marginalis, 355
Ephestia kühniella, 388, 403 ff., 416 ff., 457
Epilachna capensis, 360
— *chrysomelina*, 360
Galleria, 466, 474 ff.
— *mellonella*, 410, 472, 543
Gonepteryx rhamni, 56
Guerinia serratulae, 584
Hemimerus, 61
Hesperocterus, 61
— *fumarius*, 60
Lasius, 460
Laspeyresia pomonella, 417
Leptinotarsa, 457, 642
— *decemlineata*, 50
Lina, 466
Litomastix, 228
Locusta migratoria, 37
— *pardalina*, 36
Lomechusa, 310
Loxostege sticticalis, 583
Lucilia, 57
— *sericata*, 49 ff., 57, 472
Lygus pabulinus, 108
Lymantria, 317, 467, 472 ff.
— *dispar*, 37
— *monacha*, 37, 583
Macrosiphum solanifolii, 118
Malacosoma americana, 50
Melanoplus, 34, 37, 456, 476, 496, 626, 642
— *bivittatus*, 56
— *differentialis*, 27, 37, 49 ff., 463, 578 ff.,
Melolontha vulgaris, 56
Micronematus gallicola, 107
Murgantia histrionica, 583
Musca carnaria, 57
— *domestica*, 57
Notostira, 38
— *erratica*, 37
Oöencyrtus johnsoni, 583
Orasema, 468
Papilio centralis, 392
— *machaon*, 392
— *sphyrus*, 392
Philosamia cynthia, 403
Phormia regina, 472
Phragmatobia, 456
Phryganidia californica, opp. 464
Pieris brassicae, 456, 655
Platycnemis, 457 ff.
Platygaster, 228
Plesiocoris rugicollis, 108
Plodia interpunctella, 417
Plusia chrysitis, 417
Popillio, 475
— *japonica*, 37, 472
Pyrrhochoris, 456
Reduvius, 583
Rhabdophaga rosaria, 107
Rhodites rosae, 107
Rhodnius prolixus, 56, 464
Sceliphron caementarium, 475, 583
Sialis, 457, 466 ff.
Sitona, 460
Sminthurus viridis, 36
Spathegaster (*Neuroterus*) *baccarum*, 107
Sphodromantis, 377
Tachycines, 457
Tenebrio molitor, 377, 460, 472, 475, 543
Tineola tinella, 466
Triatoma, 464
Tribolium confusum, 466
Vanessa, 466 ff.
— *io*, 403
— *urticae*, 403
Vespa crabro, 56
Wohlfahrtia vigil, 56

MOLLUSCA

(*a*) GASTROPODA

Achatina fulica, 29
Agriolimax, 350

cylastrum fluviatilis, 59
sia limacina, 134, 135 ff., opp. 146, 576,
4
thynia tentaculata, 59
ccinum undatum, 59
imulus sporadicus, 58, 59
clostoma elegans, 58 ff.
bbula umbilicalis, 59
licella itala, 58, 59
lix, 54, 58 ff., 355
— *pomatia*, 29, 58 ff.
mifusus tuba, 29, 39, 585, 626
drobia jenkinsi, 59
mnaea, 39, 59, 75, 350, 366, 422
— *peregra*, 59
— *stagnalis*, 59, 576
ttorina irrorata, 543
— *littorea*, 58 ff.
— *neritoides*, 59 ff.
— *obtusata*, 59
— *rudis*, 59 ff.
ccella (*Purpura*) *lapillus*, 29, 59
chidella celtica, 59
ludina (*Viviparus*) *fasciatus*, 59 ff.
ysa, 131, 422
anorbis corneus, 59

(*b*) Lamellibranchiata

odonta, 29, 51, 54
arnea candida, 572
mingia tellinoides, 563
cten, 30

(*c*) Cephalopoda

loteuthis, 39
ligo, 39
— *bleekeri*, 38, 626
— *vulgaris*, 38, 49 ff.
ia, 34, 38 ff., 514
— *officinalis*, 38, 49, 543, 619
ietta, 39
iola, 39

VERTEBRATA

(*a*) Cephalochorda

nphioxus, 322, 574

(*b*) Tunicata

cidiella, 134
— *scabra*, 321
ona, 134 ff., 321, 575
lavellina, 136, 525 ff.
erophora, 527
hallusia, 134
tyela, 131, 321

(*c*) Cyclostomata

mpetra, 321
— *fluviatilis*, 322, 585
— *planeri*, 171, 325

(*d*) Pisces

(i) Elasmobranchii

Acanthias, 34, 40, 42
— *blainvillei*, 40 ff., 44
— *blanchardi*, 543
— *vulgaris*, 40 ff., 44
Carcharias glaucus, 34, 41 ff.
Centrophorus, 42, 45, 47
— *granulosus*, 41, 44 ff.
Cetorhinus maximus, 45
Chiloscyllium griseum, 27
Galeus canis, 40 ff., 44 ff.
Heptranchias deani, 27
Mustelus, 27, 42 ff.
— *antarcticus*, 40 ff., 44
— *laevis*, 39 ff., 46
— *vulgaris*, 39 ff., 44, 543
Myliobatis, 40, 42
— *bovina*, 40 ff.
Raia, 27
— *batis*, 27, 45
— *oxyrhincus*, 26
Rhinobatus panduratus, 44
Scyllium, 27, 34, 38, 40, 548, 634
— *canicula*, 39 ff., 323, 543
— *stellare*, 45
Scymnus, 40, 42, 47
— *lichia*, 41, 44
Squatina, 44
Torpedo, 38, 40 ff.
— *marmorata*, 41, 44, 543
— *ocellata*, 41, 44, 50, 543
Trygon, 34, 40 ff., 46
— *violacea*, 40 ff.

(ii) Teleostei

Alosa pontica, 45
Amblyopsis, 225
Anguilla, 54
Anoptichthys, jordani, 225
Arius falcarius, 47
Aspredo laevis, 46
Brachydanio rerio, 512
Carassius auratus, 75, 653
Chologaster, 225
Coregonus, 329
— *clupeaformis*, 326
— *lavaretus*, 362
Ctenolabrus, 509, 585
Cynoscion nebulosus, 25
Cyprinus, 54
— *carpio*, 45, 49, 543
Danio rerio, 171, 350
Esox, 54
— *lucius*, 75
Fundulus, 323, 326, 329, 351, 384 ff., 606
— *heteroclitus*, 230, 325 ff., 584
Gambusia holbrooki, 46, 66, 329
Gasterosteus aculeatus, 75
Girardinus, 46
Goodea bilineata, 46

Heterandria formosa, 46
Hippocampus, 47, 56
 — *antiquorum*, 47
 — *brevirostris*, 47, 586
Hoplias, 75
Kurtus gulliveri, 46
Lucifuga, 225
Merliccius, 26
Nerophis ophidion, 586
Oryzias latipes, 26, 46, 74, 225
Parabrotula dentiens, 46
Perca fluviatilis, 280, 325, 584
Phycis, 26
Platypoecilus maculatus, 387, 442
 — *variatus*, 387
 — *xiphodium*, 387
Pleuronectes platessa, 49 ff., 54
Poecilia, 46
Pseudopleuronectes, 387
Pteroplatea micrura, 40 ff.
Salmo, 33 ff., 38, 54, 291, 322
 — *fario*, 171, 362, 543
 — *irideus*, 49 ff., 323, 325, 584
 — *salar*, 26, 38, 49, 543, 618
Savelinus fontinalis, 49, 585
Scomber, 26, 54
Sebastes marinus, 46
Serranus, 326
Silurus glanis, 545, 624
Smaris alcedo, 26
Syngnathus acus, 47
 — *typhle*, 47
Trachycorystes, 75
Troglichthys, 225
Typhlichthys, 225
Zoarces viviparus, 46

(iii) Dipnoi

Lepidosiren paradoxa, 586

(*e*) AMPHIBIA

(i) Urodela

Amblystoma, 38, 190, 342, 344 ff., 666
 — *maculatum*, 587
 — *mexicanum*, 199 ff., 455
 — *opacum*, 34 ff.
 — *punctatum*, 33, 292, 299, 344 ff.
 — *tigrinum*, 344 ff., 350 ff., 450, 455
Cryptobranchus, 241, 617, 625, 636, 642
 — *alleghaniensis*, 641
 — *japonicus*, 39, 49 ff., 241, 641
Hynobius, 451, 617, 625, 635 ff., 641 ff.
 — *lichenatus*, 219
 — *nebulosus*, 39, 50, 192, 626
 — *unnangso*, 297
Megalobatrachus maximus, 241
Molge, 242
 — *vulgaris*, 171
Necturus, 450
Pleurodeles waltlii, 292
Proteus anguineus, 225
Salamandra, 434, 439
 — *atra*, 47
 — *maculosa*, 360 ff.
Siredon, 434
Spelerpes, 225
Triton, 156, 241, opp. 274, 306, 342, 431
 439 ff.
 — *alpestris*, 141, 193, 197, 292, 360
 — *cristatus*, 141, 148, 203, 241, 292, 3
 358 ff., 376, 543
 — *palmatus*, 358 ff., 376
 — *pyrrhogaster*, 304, 340
 — *taeniatus*, 148, 171, 292, 299, 341 ff., 357
 376
 — *torosus*, 171, 350 ff., 587
 — *virescens*, 25
Typhlomolge, 225
Typhlonectes compressicauda, 47
Typhlotriton, 225

(ii) Anura

Alytes, 305
 — *obstetricans*, 33
Bombina, 617
 — *bombina*, 292
Bombinator, 156, opp. 274, 292 ff.
 — *igneus*, 543
 — *pachypus*, opp. 290, 292, 342, 348
Bufo, 250, 355, 588, 604, 617
 — *cognatus*, 147
 — *formosus*, 221
 — *marinus*, 39
 — *viridis*, 293, 356, 587
 — *vulgaris*, 293, 357, 587
Chiroleptes platycephalus, 35
Discoglossu[illegible] 191, 588
 — *pinctus*, 147, 193, 199, 321
Eleutherodactylus, 448
Heleioporus pictus, 35
Hyla, 34 ff., 306
 — *evansi*, 48
 — *goeldii*, 48
 — *regilla*, 221
 — *rubella*, 35
Hylambates brevirostris, 48
Hylodes martinicensis, 32 ff.
Kaloula borealis, 35
Limnodynastes ornatus, 35
Natalobatrachus bonebergi, 35
Nectophrynoides tornieri, 47
Nototrema, 48
 — *marsupiatum*, 47 ff.
 — *oviferum*, 47 ff.
Pelobates, 305, 441, 449
 — *fuscus*, 292, 348
Phyllomedusa, 35
Pipa americana, 48
 — *dorsigera*, 48
Polypedates reinwardtii, 35
Protopipa aspera, 48
Rana, 34, 451, 617
 — *arvalis*, 221, 348
 — *catesbiana*, 292, 589

na clamitans, 443
esculenta, 221, 241, opp. 290, 291 ff., 317, 342, 356
fusca, 193, 221, 291 ff., 419
nigromaculata, 191
— *palustris*, 291 ff.
— *pipiens*, 221, 240 ff., 317, 589
— *sylvatica*, 193, 291, 587
— *temporaria*, 49 ff., 140, 192 ff., 197, 221, 317, 357, 543, 587
acophorus schlegeli, 35
inoderma darwini, 48

(*f*) REPTILIA

(i) Rhyncocephala

tteria, 36
henodon, 36, 516

(ii) Sauria

phisbaena, 225
nisonia superba, 61
ernia cunninghami, 61
certa, 55
— *agilis*, 241
— *viridis*, 543
opeltis vernalis, 590
gosoma, 61
lias, 280
thon sebae, 55, 241
ineura, 225
eloporus undulatus, 36
ps chalcides, 61
liqua scincoides, 61
opidonotus natrix, 34, 35, 55, 635
pinambis teguixin, 241

(iii) Chelonia

aretta caretta, 24, 35
helonia mydas, 241
mmys leprosa, 323
ys, 55, 516
laclemys centrata, 35, 38
temys geoffroyana, 241
Testudo graeca, 55
Thalassochelys corticata, 24, 34, 49 ff., 543, 620

(iv) Crocodilia

Crocodilus porosus, 241

(*g*) AVES

Buteo buteo, 543
Collocalia, 75
Columba, 63
— *livia*, 543
Corvus corone, 543
Cypselidae, 75
Gallus, 34, 63
— *domesticus*, 3 ff., 49 ff., 62, 171, 543, 591
Meleagris americana, 543
Podiceps, 34 ff.
Pterocles, 36
Scardafella inca, 393
Spilopelia, 63
Streptopelia, 63
Turdus merula, 543
Turtur, 63
Zenoidura, 63

(*h*) MAMMALIA

(i) Prototheria

Echidna, 56
Ornithorhŷncus, 56

(ii) Metatheria

Balaenoptera, 34
Canis vulgaris, 543
Cavia, 280
Dasypus novemcinctus, 228
— *villosus*, 313
Felis vulgaris, 543
Homo sapiens, 34, 543
Lepus cuniculus, 543
Mus norvegicus, 543
— *vulgaris*, 543
Sciurus niger, 647
Sus scrofa, 543

INDEX OF PLANTS

Acetabularia, 417
  — *mediterranea*, 418
  — *wettsteinii*, 418
Allium cepa, 421
Avena, 212
Azotobacter, 515

Bacillus coli, see *Escherichia*
  — *influenzae*, 611
  — *megatherium*, 512
  — *pyocyaneus*, 568
Baptisia tinctoria, 657

Chlamydomonas eugametos, 216

Equisetum, 668
Escherichia coli, 515

Fucus, 669 ff.
  — *vesiculosus*, 563

Mercurialis perennis, 657

Phaseolus, 109 ff.
  — *grandidentata*, 109
Physarum polycephalum, 672
Pseudomonas tumefaciens, 109

Rhizobium radicola, 109
Rosa, 107

Salix alba, 107
  — *caprea*, 107
  — *cinerea*, 107
  — *humilis*, 107
Strophanthus hispidus, 250

Zea mais, 421

INDEX OF GENES

aemic, 378
tlered, 399
ricot, 40
stapedic, 377 ff.

r, 394 ff., 402
dex, 400
ck, 407

rry, 412
ubby, 379
abar, 408 ff.
ret, 410
ourless, 398
rnish lethal, 428
eper, 24, 361, 376, 379, 382 ff., 428
ppled, 377

a, 388
haete, 398 ff.
mpy, 400
arf, 379, 390, 408

ony, 407
physemic, 388
ithelial, 378
eless, 376, 402
eless-2, 397
eless-4, 397

xed-tail, 374
zle, 24, 389 ff.

giant, 379
glass-2, 397
glassy (*eggshell*), 7
grey-lethal, 375, 388

hairless, 378
half-x, 368
honey, 412
hooded-lethal, 391

infrabar, 395

lethal-7, 385
lethal giant, 361
lobe-c, 397
lozenge-3, 397

miniature, 400
mottled (*eggshell*), 7
mottled-eye, 402

naked, 378
nicked, 399 ff.
notch-8, 368
notched, 399 ff.
no-wing, 399 ff.
nullo-IV, 367
nullo-X, 367

proboscipedic, 378
purple, 412

ragged, 399
red, 398
ruby, 408
rudimentary-12, 379
rumpless, 375

scute, 402
sepia, 408, 412
shaker, 390
shaker-short, 373 ff.
short-spine, 375
short-tail, 361, 367, 374
short-wing, 402
snipped, 402
star, 367
sticky, 388
strap, 399
stubble, 474
stubbloid, 474

thread, 378

ultrabar, 395 ff.

vermilion, 398, 408 ff.
vestigial, 379, 398 ff.

watery white, 8
white, 412 ff.

yellow, 361, 382, 407

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